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Editors-in-Chief: Renato Deláscio Lopes, José Maria Soares Jr and Roseli Nomura.
Managing Editor: César Teixeira
E-mail: ramb@amb.org.br
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ADDRESS: Rua São Carlos do Pinhal, 324
Bela Vista – São Paulo
Postal Code: 01333-903
Phone no.: (+55 11) 3178-6800 Ext. 177
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Melatonin, menopause, and thyroid function in gynecologic endocrinology: what is the role?

José Maria Soares Junior[1], Dzemail Detanac[2], Ilker Sengul[3,4], Stefan Dugalic[5], Demet Sengul[6*], Dzenana Detanac[7]

Melatonin (MTN), a neurohormone primarily synthesized and secreted mainly by the pine cone-shaped gland of the cerebrum, named as the conarium or epiphysis cerebri, from amino acid tryptophan, was first isolated from the bovine pineal gland by Lerner et al[1]. However, the 17th-century philosopher René Descartes hypothesized that the pineal gland of the brain, which remains poorly understood to date, represents the location of the *homo sapiens* soul; paleontologists described it as an ancestral "third eye"; and modern psychology declares perception beyond physical visual function[2,3]. This chemical messenger, *per se*, ensures high precision in the reconnoitering of the night period, is an endocrine marker for darkness, and participates in the regulation of circadian rhythm and the sleep-wake cycle. Of note, MTN can be produced by other organs, such as the brain, lungs, gastrointestinal tract, liver, thyroid, and reproductive and immune systems, and is present in mucus, saliva, breast milk, urine, sperm, amniotic fluid, Graafian follicle, etc.[1,4-9]. As such, various studies have shown that MTN affects many functions in the body and acts on different tissues, and some of its properties include significant antioxidant, anti-inflammatory, antiproliferative, and immunomodulatory capacity. MTN, *per se*, can directly neutralize toxic free radicals more effectively, suppresses chronic oxidative stress, has a significant impact on reproductive cells, enhances the quality of sperm and oocytes, has oncostatic and antitumoral cytoprotective effects[10,11], alleviates some of the undesirable toxic effects of radiotherapy and chemotherapy by increasing the tolerance of healthy tissues, compared to other antioxidants, by stimulating responses to DNA damage. To this end, MTN's significant role in some chronic diseases, such as diabetes, blood glucose level regulation, and hypertension, has also been reported. MTN has been studied as a therapeutic option for many autoimmune diseases of multiple sclerosis, rheumatoid arthritis, and diabetes mellitus, based on its immunoregulatory properties. MTN achieves its effect through its receptors type 1 and type 2 (MT1 [$Mel1_a$] and MT2 [Mel_{1b}]) membrane-bound receptors. Moreover, the presence of the MT1 receptor in the thyroid gland has been proven, which indicates the possibility of MTN's influence on thyroid activity and hormone production. A third membrane-bound MTN-binding site, the MT3 receptor, was theorized as a biological target of MTN and was found to, in fact, act as the cytosolic enzyme, quinone reductase II (NQO2)[12]. Some authors have reported in their genetic study that the single-nucleotide polymorphism of MTN receptor type 1A, MTNR1A, coding the MT1 protein, was associated with a sensitivity to Graves' disease and thyroid autoantibody formation, which supports the impression, that MTN can influence the development of autoimmune thyroid disease in thyroidology[13,14]. The thyroid gland is characterized by a high level of oxidative stress, and the use of pro-oxidants can lead to miscellaneous damage and diseases of this delicate papillon gland. Excessive iodine load, as an exogenous pro-oxidant, can induce apoptosis in the thyroid gland follicular cells. Furthermore, iodine compounds used in iodine prophylaxis also have potentially harmful effects. The thyroid gland is less sensitive to the pro-oxidative effects of potassium iodate, KIO_3, and reacts more strongly to the antioxidant effect of MTN than other tissues, which plays a significant role in

[1]Universidade de São Paulo, Faculdade de Medicina, Hospital das Clínicas, Departamento de Obstetrícia e Ginecologia, Disciplina de Ginecologia, Laboratório de Ginecologia Estrutural e Molecular, São Paulo (SP), Brazil.
[2]General Hospital Novi Pazar, Department of Surgery – Novi Pazar, Serbia.
[3]Giresun University, Faculty of Medicine, Division of Endocrine Surgery – Giresun, Turkey.
[4]Giresun University, Faculty of Medicine, Department of General Surgery – Giresun, Turkey.
[5]University Clinical Center of Serbia, Department of Gynecology and Obstetrics, Clinic for Gynecology and Obstetrics – Belgrade, Serbia.
[6]Giresun University, Faculty of Medicine, Department of Pathology – Giresun, Turkey.
[7]General Hospital Novi Pazar, Department of Ophthalmology – Novi Pazar, Serbia.
*Corresponding author: demet.sengul.52@gmail.com
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this phenomenon. Hence, MTN should be considered to avoid the potential damaging effects of iodine compounds applied in iodine prophylaxis[15-19]. Radiotherapy for head and neck tumors can often damage the thyroid follicular structure even though it is not affected by the tumor. However, administration of MTN before radiotherapy might attenuate the degree of tissue damage. Some authors pointed out the radioprotective effects of MTN in the acute phase of thyroid tissue damage. Besides the aforementioned characteristics, MTN has a crucial role in regulating human reproduction processes, such as oocyte quality, folliculogenesis, oocyte maturation, embryo implantation, fetal development, and the outcomes of pregnancy. Therefore, the idea of MTN utilization in the therapeutic approaches of reproductive and gestational disorders seems favorable to some authorities[20,21]. The low MTN levels in elderly people are correlated with reproductive aging and high gonadotropin secretion, while menopause is characterized by the inability of the ovaries to produce viable follicles and hormonal changes, which leads to menstrual cycle failures. Ovarian aging is characterized by reduced follicular reserve and augmented gonadotropin secretion[22]. In menopause, various alterations emerge in a woman's body, which results in the changes in her mental and physical health statuses. Anecdotally, women experience immuno-metabolic fluctuations, such as hormonal perturbations, sleep problems, and vasomotor symptoms, during menopause. Since MTN is involved in all these processes, it has the potential as a medication with multiple health benefits for the management of a menopausal woman[23,24]. In addition, some authors stated that MTN usage can improve physical symptoms such as sleep quality, mood state, estradiol levels, and body mass index in a menopausal woman, but not in the general menopausal ones[22]. In summary, MTN appears to play a key role in the regulation of the endocrine system, involving the regulation of gonadotrophin-releasing hormone (GnRH), promotion of progesterone synthesis, stimulation of oxytocin secretion, regulation of cortisol production, and promotion of androgen generation[12]. Nevertheless, it is critical to distinguish between the physiological effects and the pharmacological consequences of MTN administration due to specific differences in the response of any tissue to the agent and to determine whether the stage of development, sex differences, and genetic variability can affect how reproductive tissue responds to MTN by gynecologic endocrinologists and thyroidologists. As a matter of fact, this issue merits further investigation.

AUTHORS' CONTRIBUTIONS

JMSJ: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **Dzemail D:** Investigation, Project administration, Validation, Visualization, Writing – original draft. **IS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – review & editing. **SD:** Investigation, Validation, Visualization. **DS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Supervision, Writing – review & editing. **Dzenana D:** Investigation, Project administration, Validation, Visualization.

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GUIDELINE

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Headaches in adults in supplementary health: management

Marcelo Cedrinho Ciciarelli[1], Caio Vinicius de Meira Grava Simioni[2], Renata Gomes Londero[3*]

The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct research and to assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical condition of each patient.
Societies: Brazilian Academy of Neurology

DESCRIPTION OF THE EVIDENCE COLLECTION METHOD

Research strategy on headache treatment: a search was carried out in PubMed, LILACS, and SciELO with the following search strategy: headache (Mesh Terms) AND treatment (Mesh Terms). With the strategy headache and treatment, or migraine and treatment, the Cochrane secondary database was searched. This initial search, restricted to publications from the past 20 years, resulted in 35,112 articles. Filters were then used for articles published in Portuguese and English, randomized clinical trials (RCTs), and guidelines, resulting in 9782 articles. Excluding articles on the treatment of secondary headaches, cranial neuralgias, and primary headaches other than migraines, tension-type headaches, and cluster headaches, 85 articles were selected for use in preparing this clinical guideline. Inclusion criteria: adult or elderly patients (studies on the pediatric population were excluded), with clinical complaints of headache, with diagnoses of a primary headache compatible with the diagnoses prevalent in the clinic; preferably RCTs, but, in the absence of these for the specific topic, nonrandomized, comparative studies between drugs (not placebo) were included; series and case reports were excluded whenever there was better evidence available; and articles with internal validity and potential external validity for Brazilian reality were included. Exclusion criteria: articles that focus on realities different from the Brazilian one (medicines not available in Brazil); articles in which the internal validity could be questioned; articles aimed at the management of secondary headaches (except for medication overuse headache); and articles whose treatment focus was not medication (manipulation, cognitive behavioral therapy, and others).

DEGREES OF RECOMMENDATION AND STRENGTH OF THE EVIDENCE

- A: Experimental or observational studies of better consistency.
- B: Experimental or observational studies of lower consistency.
- C: Case reports or case series (uncontrolled studies).
- D: Opinion devoid of critical assessment, based on consensus, experts, physiological studies, or animal models.

GOALS:

This study aimed to evaluate the updated what would be the best therapeutic approach for the complaining of headache in adult patients treated in supplementary healthcare (electively), considering the most prevalent diagnoses and the best evidence available to support the approach.

INTRODUCTION

Headache is the most prevalent neurological condition and the third-most common painful reason for seeking medical care. In all, 50% of the world's population will have at least one headache attack per year, and more than 90% will have one

[1]Universidade Barão de Mauá, Brazilian Academy of Neurology, Faculty of Medicine – São Paulo (SP), Brazil.
[2]Universidade de São Paulo, Brazilian Academy of Neurology, Clinical Hospital, Faculty of Medicine – São Paulo (SP), Brazil.
[3]Brazilian Academy of Neurology, Porto Alegre Clinical Hospital – Porto Alegre (RS), Brazil.
*Corresponding author: rlondero@hcpa.edu.br
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in their lifetime. The average lifetime prevalence of migration is 18%, and the estimated average prevalence during the last year was 13%. Tension-type headache is more common than migraine (lifetime prevalence of approximately 52%), but, as it is less disabling, it less frequently leads the patient to consult.

Tension-type headaches and migraines are frequent causes of absenteeism and presenteeism, with occasional (as a group) being the second-most common cause of years lost due to disability in the world: 7.2 and 44.5 million years lost due to disability in 2015, respectively.

Despite the impressive numbers, it is estimated that more than 70% of people with recurrent headaches in the world do not receive adequate diagnosis and management.

CLINICAL ISSUES

What is the best treatment to end a current headache attack – symptomatic treatment of the attack?

What is the best treatment to prevent recurrent headaches – prophylactic treatment of different primary headaches?

GUIDELINES FOR THE ACUTE SYMPTOMATIC MANAGEMENT OF PRIMARY HEADACHES

Symptomatic treatment of primary headaches aims to reduce the intensity or eliminate pain in a sustainable, safe, and accessible way. Correct guidance on the treatment of crises provides functional recovery, avoiding the need for emergency services and reduced work capacity. It is important, however, to raise awareness of the rational use of acute symptomatic medications, as their indiscriminate use can lead to medication overuse headache, a complicating factor in primary headaches[1].

Staggered x stratified treatment[2]

The choice of acute symptomatic treatment can be made in the following way:

- Staggered: treatment begins with the prescription of nonspecific analgesic drugs. At each consultation, the doctor can adjust the symptomatic medication according to the response obtained, taking into account the results of the previous prescription.
- Stratified: the doctor, based on the description of the crisis and the patient's previous experience, prescribes a treatment that would be compatible with their intensity and response to treatments already tried.

There is evidence that stratified treatment is more effective in reducing the time for pain relief, the recurrence of attacks, and the need for additional doses of medication, which is why it is recommended in this guideline.

Acute symptomatic treatment of migraine

The treatment of migraine attacks must be based on individual aspects, since migraine is a complex disease with multiple characteristics that vary from one person to another, which can influence the outcome of treatment. Drugs must be chosen taking into account each patient's history (previous results, allergies, contraindications, and comorbidities)[1].

Nonspecific and/or specific drugs can be used[3]. Specific drugs are triptans and ergot derivatives. Nonspecific drugs are simple analgesics and nonhormonal anti-inflammatory drugs (NSAIDs). The combined use of antiemetics, neuroleptics, and corticosteroids may be necessary. Opioids, however, should be avoided[4]. For doses, route of administration, and grade of recommendation for the use of different medications, see Tables 1 and 2. For the main studies that supported the recommendation, see Table 3.

Acute symptomatic treatment of migraine during pregnancy/lactation

Pregnancy can cause changes in the previous pattern of migraine. A reduction in the frequency and intensity of attacks, as well as a faster response to symptomatic medications, usually occur during pregnancy. Less frequently, the remission, worsening, or even onset of migraine attacks for the first time may be observed.

This treatment guideline emphasizes pharmacological measures and their respective levels of evidence; however, it is important to highlight that during pregnancy and lactation, preference is given to nonpharmacological measures, particularly for less intense painful episodes. If there is a need for drug treatment, it is always necessary to evaluate the relationship between risk and benefit for the fetus. However, the weak scientific evidence related to maternal-fetal efficacy and safety must be taken into account.

Acute symptomatic treatment of tension-type headache

Most tension-type headache attacks are mild to moderate in intensity, so patients often self-medicate with simple analgesics (e.g., paracetamol or acetylsalicylic acid) or NSAIDs. The effectiveness of simple analgesics tends to decrease with increasing headache frequency.

Even so, simple analgesics[5] and NSAIDs[6] are the main treatments for tension-type headache attacks. Paracetamol

Table 1. Medications used in migraine attacks: dose, route of administration, and grade of recommendation.

Medication	Dose	Route of administration	Grade of recommendation
Paracetamol	1000 mg	PO	A
Dipyrone	1000 mg	PO	B
Naproxen	500/550 mg	PO	A
Ibuprofen	200/400 mg	PO	A
Diclofenac	50/100 mg	PO	A
Acetylsalicylic acid	500 mg	PO	A
Naratriptan	2.5 mg	PO	A
Rizatriptan	5 mg	PO	A
Sumatriptan	25/50/100 mg	PO	A
	10 mg	NASAL	
	6 mg	SC	
Zolmitriptan	2.5 mg	PO	A
Sumatriptan/naproxen	85/500 mg	PO	A
Paracetamol/acetylsalicylic acid/caffeine	500/500/300 mg	PO	A
Chlorpromazine	12.5 mg	IM	B
Metoclopramide	10 mg	IV	B
Ketoprofen	100 mg	PO	B
Ketorolac	30/60 mg	IV/IM	B
Magnesium sulfate (migraine with aura)	1–2 g	IV	B
Dexamethasone	4–16 mg	IV	C

Table 2. Medications used to treat migraine attacks: reviewed studies and grade of recommendation.

Medication	Author, year (n)	Result	Grade of recommendation
Acetaminophen	Freitag, 2008 (173)[3], Prior, 2010 (346)[17]	Acetaminophen superior to placebo	A
Dipyrone	Bigal, 2001 (269)[7]; Bigal, 2002 (74)[18]	Dipyrone superior to placebo	B
Acetylsalicylic acid	Lipton, 2005 (485)[19], MacGregor, 2002 (101)[20]	Acetylsalicylic acid superior to placebo	A
Ibuprofen	Codispoti, 2001 (660)[21], Diener, 2004 (312)[22]; Misra, 2007 (124)[23]	Comparable to sumatriptan and acetylsalicylic acid+metoclopramide; superior to placebo, inferior to zolmitriptan	A
Naproxen	Nestvold, 1985 (41)[24], Johnson, 1985 (70)[25]; Wentz, 2008 (337)[26], Smith, 2005 (972)[27]	Superior to placebo	A
Sumatriptan	Smith, 2005 (972)[27]; Bussone, 2000 (233)[28]	Superior to placebo	A
Sumatriptan + naproxen	Smith, 2005 (972)[27]	Superior to sumatriptan alone, naproxen alone, placebo	A
Rizatriptan	Freitag, 2008 (173)[3], Seeburger, 2011 (102)[29]	Superior to placebo and paracetamol; superior to placebo in nonresponders to sumatriptan	A
Zolmitriptan	Misra, 2007 (124)[23]	Superior to ibuprofen and placebo	A

Table 3. Medicines, nutraceuticals, and devices used in the prophylaxis of episodic migraine, doses, indications, and side effects.

Medication	Starting dose	Maintenance dose	Additional beneficial effects	Side effects	Grade of recommendation
Propranolol[36]	10 mg BID	80–240 mg, BID or TID	Essential tremor, heart rate control, antihypertensive	Tiredness, asthma exacerbation, decreased libido, depression, increased triglycerides	A
Metoprolol succinate[37]	25 mg qd	100–200 mg qd	Heart rate control, antihypertensive	Decreased libido, depression, increased triglycerides Better tolerated than propranolol	A
Topiramate[38,39]	25 mg at night	25–100 mg at night or BID 25 mg increase every 4 weeks	Reduction of body weight, indicated for headache secondary to idiopathic intracranial hypertension, mood stabilizer, anti-epileptic	Contraindicated during pregnancy; interacts with contraceptives, which may reduce their effectiveness	A
Valproato[40]	250 mg 12/12 hours	500–1500 mg BID	Mood stabilizer, anti-epileptic	Contraindicated during pregnancy, avoid in women at risk of pregnancy, weight gain, hair loss	A
Divalproato[41,42]	250 mg qd	250–1500 mg qd	Mood stabilizer, anti-epileptic	Contraindicated during pregnancy, avoid in women at risk of pregnancy	A
Atenolol[43]	25 mg qd	50–200 mg qd	Heart rate control, greater antihypertensive effect	Decreased libido, depression, increased triglycerides Better tolerated than propranolol	B
Amitriptilina[38]	10 mg at night	10–200 mg at night	Improves sleep, antidepressant, in comorbidity with tension-type headache	Constipation, dry mucous membranes, palpitation	B
Nortriptilina*[44]	10 mg at night	10–200 mg at night	Antidepressant, in comorbidity with tension-type headache	Constipation, dry mucous membranes, palpitation (less common than with amitriptyline)	–
Venlafaxine[45,46]	37.5 mg qd	75–225 mg qd	Management of depression, anxiety, changes in sleep.	Weight loss, nausea, vomiting.	B
Candesartan[47]	8 mg in the morning	8–16 mg daily	Antihypertensive Indicated as an adjuvant	Contraindicated during pregnancy	C
Lisinopril[48]	5 mg in the morning	5–10 mg in the morning	Antihypertensive Indicated as an adjuvant	Contraindicated during pregnancy	C
Gabapentina[49]	300 mg at night	300–1800 mg 12/12 hours	–	–	U
Verapamil[50]	40 mg BID	180–480 mg BID	Heart rate control	Lower limb edema	U
Flunarizine[36,51]	5 mg at night	5–10 mg at night	Anti-vertigo effect, improves sleep	Weight gain, drowsiness, parkinsonism with prolonged use	–
Eptinezumab***[52]	100 mg IV	100–300 mg IV	–	High cost, need for application in hospital environment	A
Erenumab**[53,54]	70 mg	70–140 mg/month SC	–	High cost	A
Fremanezumab[55,56]	225 mg/month SC 675 mg/3 months SC	225 mg/month SC 675 mg/3 months SC	–	High cost	A
Galcanezumab[57]	240 mg/month - 1ª dose	120 mg/month SC	–	High cost	A
Magnesium[58]	400–600 mg qd	400–600 mg qd	Safe during pregnancy and breastfeeding Has an effect on constipation	Diarrhea	B
Coenzyme Q-10[59,60]	300 mg qd	300 mg qd	Safe during pregnancy and breastfeeding	–	C
Riboflavin[60,61]	400 mg qd or 200 mg BID	400 mg qd or 200 mg BID	No side effects	Because it is used at a dose above the physiological level, the safety of use during pregnancy is still under discussion	B
Electrical stimulation of the supraorbital nerve[62]	Specific protocol	20 min, once a day	Safe during pregnancy and breastfeeding	Discomfort at the site: from paresthesia to a slight sensation of shock	B

qd: single dose; Grade A evidence: established efficacy; grade B: probable efficacy; grade C: possible efficacy; grade U: inadequate data or conflicting evidence, (–) with no degree of evidence defined to date. *Medication widely used when amitriptyline causes drowsiness, but without studies proving effectiveness. **At the time of publication, approved for use by ANVISA but no longer available for sale. ***At the time of publication, approved for use by ANVISA but not available for commercialization.

is less effective than NSAIDs but has fewer gastric adverse effects. Combinations with caffeine-containing analgesics are more effective than simple analgesics and NSAIDs[6]; however, they increase the risk of headaches due to excessive medication use. Triptans, myorelaxants, and opioids are not indicated for the acute symptomatic treatment of tension-type headaches. Medications, doses, and grade of recommendation are presented in Table 4.

Acute symptomatic treatment of cluster headache

Cluster headache attacks are considered the most serious among primary headaches due to their very intense intensity, association with autonomic symptoms, and high daily frequency. Furthermore, a reasonable proportion of patients with cluster headaches have the chronic form of the disease, characterized by short periods or lack of remission. Subcutaneous sumatriptan[13] and mask oxygen inhalation[14,15] remain at recommendation grade A. The form of prescription for these, recommendation grade, and other drugs also prescribed for the condition are presented in Table 5.

The transitional treatment with the best recommendation grade, B, consists of anesthetic block of occipital nerves with corticosteroids[16].

Table 4. Oral medications used to manage tension-type headache attacks: dose and grade of recommendation.

Medication	Dose (PO)	Grade of recommendation
Dipyrone (Metamizol)[5,7]	500–1000 mg	A
Ibuprofen[8,9]	200–400 mg	A
Ketoprofen[9]	25–50 mg	A
Acetylsalicylic acid[5]	500–1000 mg	A
Naproxen[9]	375–550 mg	A
Diclofenac[10]	12.5–100 mg	A
Paracetamol[11]	1000 mg	A
Combinations with caffeine[6,12]	65–200 mg	B

GUIDELINES FOR THE PROPHYLACTIC MANAGEMENT OF PRIMARY HEADACHES

Episodic migraine prophylaxis

Defining episodic migraine: it is characterized by migraine that occurs between 3 and 14 days per month in the last 3 months.

For those who prescribe prophylactic therapy: Any patient with migraine who presents with headache 4 or more days per month or 8 or more days with headache in the last 3 months is a candidate for prophylactic treatment. Beta blockers, tricyclic and dual antidepressants, and anticonvulsants are usually used. Additionally, there are non-drug methods indicated for prophylaxis: acupuncture[30,31], biofeedback[32], cognitive-behavioral therapy, aerobic exercises, and electrical stimulation (transcutaneous electrical stimulation of the supraorbital nerve). These can be adopted in association with drug prophylaxis or as isolated therapy, in this case, especially for pregnant women, breastfeeding women, people who prefer non-drug methods, or who are intolerant of available medications.

Expected benefits for the patient who receives prophylactic treatment are as follows: (1) reduction in the number of days with pain, (2) reduction in pain intensity, (3) reduction in the duration of attacks, and (4) improvement in the response to medications used for relief of attacks (symptomatic medications). Furthermore, evidence suggests that the use of prophylactic medication can prevent the progression of migraine.

General principles of migraine prophylaxis (see Table 6), adapted from Dodic (2018)[33]: the drug is chosen taking into account comorbidities, associated diseases, medications previously used by the patient, and a pregnancy plan. Medications are usually started at a low dose, with a progressive increase after subsequent reassessments, which improves tolerance to the potential side effects of medications. Use for a minimum period of 2–3 months is necessary to assess effectiveness[34]. Several groups of medications have already been tested for prophylactic use, including beta blockers, tricyclic and dual antidepressants, neuromodulators, anticonvulsants,

Table 5. Medications used in cluster headache attacks: dose, route of administration, and grade of recommendation.

Medication	Dose	Route of administration	Grade of recommendation
Sumatriptan	6 mg	SC	A
Oxygen	100% 6–12 l/min	Nasal (mask)	A
Sumatriptan	10 mg	Nasal (spray)	B
Zolmitriptan	5–10 mg	PO	B
Lidocaine	10%	Nasal (spray)	C

and CGRP inhibitors. See Table 3 for drugs, starting and maintenance doses, potential associated beneficial effects, and most prevalent evidence-based paraeffects[35].

Tension-type headache prophylaxis

Tension-type headache is very common, with a prevalence in the general population varying between 30 and 78% in different studies. Although already considered primarily psychogenic, several studies suggest a neurobiological basis for at least the most severe subtypes of tension-type headache. Tension type headache is divided into episodic and chronic types (more than 15 days per month). The episodic form was subdivided into an infrequent type (less than 12 days per year with pain) and a frequent type (12 or more to less than 180 days per year with pain). Frequent episodic tension-type headaches can be associated with considerable disability and require treatment with medications. Chronic tension-type headache is a serious illness that causes a major decline in quality of life and a high degree of disability and must invariably be managed with prophylaxis. For commonly prescribed drugs, doses, and degrees of recommendation, see Table 7.

Diagnostic criteria for episodic tension-type headache ICHD-3:[63]

A. Lasting from 30 min to 7 days
B. At least two of the following four characteristics
   1. Bilateral location
   2. Pressing or tightening (nonpulsating) quality
   3. Mild or moderate intensity
   4. Not aggravated by routine physical activity such as walking or climbing stairs
C. Both of the following:
   1. No nausea or vomiting
   2. No more than one of photophobia or phonophobia

Table 6. General principles of migraine prophylaxis.

1. Start with medication at a low dose and increase slowly – usually every 2 weeks, at least.
2. Use the medication for at least 2–3 months, except in the event of intolerable side effects.
3. Pay attention to contraindications and drug interactions.
4. Reinforce the use of the headache diary as a way of monitoring treatment.
5. Watch out for excessive use of painkillers.
6. Assess possible comorbid conditions that aggravate migraine.
7. Consider a combination of prophylactic agents from different categories for refractory patients.
8. Reduce and withdraw prophylaxis when the crises are controlled, in general for 3 months with less than 3 days of pain per month.

Diagnostic criteria for chronic tension-type headache:[1]

A. Headache occurring on ≥15 days/month on average for >3 months (≥180 days/year), fulfilling criteria B-D
B. Lasting hours to days, or unremitting
C. At least two of the following four characteristics:
   1. Bilateral location
   2. Pressing or tightening (nonpulsating) quality
   3. Mild or moderate intensity
   4. Not aggravated by routine physical activity such as walking or climbing stairs

Both of the following:

No more than one of photophobia, phonophobia, or mild nausea

Neither moderate or severe nausea nor vomiting

Not better accounted for by another ICHD-3 diagnosis.

Cluster headache prophylaxis

Defining cluster headache: cluster headache is characterized by symptoms that recur in short periods, one to eight times a day, daily, for a few weeks or months. It is characterized by sudden and intense, fixed unilateral, ocular, or periorbital pain, associated with at least one of the following: conjunctival injection and/or tearing; nasal congestion and/or rhinorrhea; eyelid edema; frontal and facial sweating; miosis and/or ptosis; a feeling of restlessness or agitation.

For which patient to prescribe prophylactic therapy: for every patient with cluster headache.

Expected benefits for the patient who receives prophylactic treatment are as follows: (1) reduction in days with pain, (2) reduction in pain intensity, (3) reduction in attack duration, and (4) improvement in the response to medications for relief of crises (symptomatic medications).

General principles of cluster headache prophylaxis (see Table 8): Cluster headache management includes the use of acute medications for the attack (see a specific chapter on acute management), prophylactic treatment, and transitional

Table 7. Prophylactic treatment of tension-type headache.

Drug	Dose	Grade of recommendation
Amitriptyline[64,65]	25–75 mg PO	A
Mirtazapine[66]	30 mg PO	B
Venlafaxine[45,67]	150 mg PO	B
Clomipramine[68]	75–150 mg PO	B
Maprotiline[69]	75 mg PO	B
Mianserin[68]	30–60 mg PO	B

treatment. Transitional treatment consists of prescribing medications that take effect faster than prophylactic ones but must be used for a short period of time. It is indicated in two situations: (1) as isolated prevention for patients with short cycles of pain and (2) as a "bridge" for patients with long cycles of pain while another preventive medication is adjusted. The main treatments included here are occipital nerve block (using local anesthetics associated with corticosteroids) and a course of oral corticosteroids. Blocking is usually carried out once and can be repeated within a minimum period of 3 months. The course of oral corticosteroids should last a maximum of 3 weeks and should not be repeated more than two to three times a year. Both time limitations mentioned are due to the side effects of frequent use of corticosteroids. The prophylactic treatment with the best established efficacy is verapamil. If this fails or in cases where it is contraindicated or not tolerated, the options are: topiramate and lithium, and, with less evidence (case series and expert opinions), sodium valproate, baclofen, and testosterone replacement therapy. Melatonin may also be indicated, usually as an adjunct treatment. For refractory cases, sphenopalatine ganglion block and occipital nerve stimulation are still available. Prophylaxis should be started as soon as the diagnosis is established, and slow reduction and subsequent suspension can be considered after the patient remains asymptomatic for at least two weeks.

Prophylaxis of chronic migraine associated or not with headache due to excessive use of analgesics

The current International Classification of Headaches[63] (ICHD-3) sets up a specific chapter for chronic migraine (CM) and characterizes it as pain that occurs more than 15 days a month for a period longer than 3 months without excessive use of symptomatic medications; as long as at least 8 days of the month, the pain presents typical characteristics of a migraine crisis. During the anamnesis, it is important to highlight the previous history of episodic migraine and its evolutionary nature, which is often associated with the loss of migraine characteristics (see Diagnostic criteria for chronic migraine ICHD-3 in https://ichd-3.org/1-migraine/1-3-chronic-migraine/). Chapter 8 of ICHD-3 covers pre-existing headache, which, in association with excessive use of analgesics, causes a significant worsening of pain frequency. This is characterized by a headache that occurs 15 or more days per month, and its progression was a consequence of the excessive and regular use of symptomatic medications (10 or more days with symptomatic medication, 15 or more days with symptomatic medication, depending on the medication) for a period longer than 3 months. The headache usually improves when use is stopped (see Criteria for headache attributed to medication overuse, according to ICHD-3[63], in https://ichd-3.org/8-headache-attributed-to-a-substance-or-its-withdrawal/8-2-medication-overuse-headache-moh/).

Table 8. Medications and procedures used in cluster headache prophylaxis, doses, and effects.

Medication/procedure	Starting dose	Maintenance dose	Side effects	Grade of recommendation
Verapamil[70]	80 mg BID	80–320 mg, TID	Prolongation of the T interval, tremor	A
Galcanezumab[71]	300 mg SC monthly	300 mg SC monthly	Rare: constipation	A
Oral corticosteroid[72]	Prednisone/prednisolone 1 mg/kg, in the morning	Slow reduction over 2–3 weeks	Hip osteonecrosis, lack of blood pressure and glycemic control	A
Ipsilateral greater occipital nerve block[73,74]	Lidocaine 1–2% 1–4 mL, or bupivacaine 0.25–0.5%, 1–4 mL Associated with 80 mg methylprednisolone	Lidocaine 1–2% 1–4 mL, or bupivacaine 0.25–0.5%, 1–4 mL Associated with 80 mg methylprednisolone	Pain at the application site, rare: hair loss at the application site	B
Lithium[75]	300 mg at night	900 mg/day, serum level 0.7–1.2 mmol/L	Polyuria	B
Topiramate[76]	50 mg at night	100–400 mg qd	Paresthesia, drowsiness, changes in mood, taste	B
Melatonin[77]	10 mg at night	10 mg at night	No reported adverse effects	C
Clomiphene[78,79]	300 mg for 3 days	50 mg for 45–180 days	Acne, ovarian cyst	C

The treatment of CM should always be preceded by a careful review of the diagnosis, detection of possible worsening factors and associated conditions, stratification of severity/intractability, and monitoring with a pain diary.

Regarding therapeutic measures for CM, prophylactic treatment should always be prioritized over acute treatment. If severe and disabling crises occur, analgesia should be stimulated by nonpharmacological methods.

Prophylactic pharmacological management of CM (Table 9) is always indicated. The association of CM with medication-overuse headache may require, although in a minority of cases, management in hospital. The criteria for defining this need are described in Table 10. Removing excessively used medications can be very challenging, and transitional treatment can be of great value in this case (Table 11).

Diagnostic criteria for chronic migraine ICHD-3[1]

A. Headache (migraine-like or tension-type-like) on ≥15 days/month for >3 months, and fulfilling criteria B and C.
B. Occurring in a patient who has had at least five attacks fulfilling criteria B–D for 1.1 *Migraine without aura* and/or criteria B and C for 1.2 *Migraine with aura*.
C. On ≥8 days/month for >3 months, fulfilling any of the following:
   1. Criteria C and D for 1.1 *Migraine without aura*

Table 9. Prophylactic treatment of chronic migraine.

Drug	Dose	Grade of recommendation
Onabotulinum toxin type A[80,81]	155–195 UI/cycle, repeated every 12 weeks, for at least the 2–3 cycles	A
Topiramate[38,39,82]	50–100 mg BID PO	A
Divalproex[83]	1000 mg/day PO	B
Amitriptyline[38]	10–200 mg/day PO	A
Galcanezumab[84]	120 mg/month SC	A
Fremanezumab[56,85]	225 mg/month or 675 3/3 months SC	A

Table 10. Situations to consider initial management of migraine in an inpatient setting.

Lack of response to appropriate treatment on an outpatient basis.
History of frequent visits to emergency units.
Migraine status or crisis refractory to acute treatment in the emergency unit.
Intense nausea, vomiting, or diarrhea causing dehydration, water and electrolyte disturbance, and/or preventing oral treatment. Special attention should be paid to conditions such as pregnancy, postpartum period, chronic renal failure, severe ischemic heart disease, and arrhythmias.
Changes in vital hemodynamic (blood pressure and heart rate) and respiratory (respiratory rate and O_2 saturation) data.
Need to stop the excessive use of symptomatic medications (acute analgesics and antimigraine drugs) and the treatment of manifestations related to toxicity and/or dependency/rebound phenomena that cannot be safely managed on an outpatient basis (parenteral treatment and/or intensive symptom monitoring).
Subentrant epileptic seizures or status epilepticus, severe allergic reactions, renal or hepatic failure, thrombocytopenia, bleeding, vascular insufficiency, and serious infection.
Concomitant need for psychiatric hospitalization (risk of aggression, suicide, moral exposure, severe psychosis, detoxification of drug addicts, and abstinence).
When reviewing the diagnosis, it requires procedures best performed in a hospital setting.
Presence of psychosocial factors that prevent adequate treatment outside a controlled environment.

Table 11. Transitional treatment of chronic migraine associated with headache due to excessive use of analgesics.

Discontinuation of the drug in excessive use	Treatment of rebound headache	Treatment of withdrawal symptoms
Abrupt in the case of analgesics	Try nonpharmacological measures	Antiemetics
Gradual in cases of excessive use of barbiturates, benzodiazepines, and opioids	Use of unused analgesics, limited to twice a week	Corticosteroids for 7–14 days

2. Criteria B and C for 1.2 *Migraine with aura*
3. Believed by the patient to be migraine at onset and relieved by a triptan or ergot derivative

D. Not better accounted for by another ICHD-3 diagnosis.

Diagnostic criteria for medication overuse headache, according to ICHD-3[1]

A. Headache occurring on ≥15 days/month in a patient with a pre-existing headache disorder.
B. Regular overuse for >3 months of one or more drugs that can be taken for acute and/or symptomatic treatment of headache.[1, 2, 3]
C. Not better accounted for by another ICHD-3 diagnosis.

AUTHORS' CONTRIBUTIONS

MCC: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – original draft.
CVMGS: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – original draft.
RGL: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing.

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[1]Patients should be coded for one or more subtypes of 8.2 Medication-overuse headache according to the specific medication(s) overused and the criteria for each below. For example, a patient who fulfills the criteria for 8.2.2 *Triptan-overuse headache* and the criteria for one of the subforms of 8.2.3 *Non-opioid analgesic-overuse headache* should receive both of these codes. The exception occurs when patients overuse combination-analgesic medications, who are coded 8.2.5 *Combination-analgesic-overuse headache and not according to each constituent of the combination-analgesic medication.*

[2]Patients who use multiple drugs for acute or symptomatic treatment of headache may do so in a manner that constitutes overuse even though no individual drug or class of drug is overused; such patients should be coded 8.2.6 *Medication-overuse headache attributed to multiple drug classes not individually overused.*

[3]Patients who are clearly overusing multiple drugs for acute or symptomatic treatment of headache but cannot give an adequate account of their names and/or quantities are coded 8.2.7 *Medication-overuse headache attributed to unspecified or unverified overuse of multiple drug classes* until better information is available. In almost all cases, this necessitates diary follow-up.

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Microglia role in the pain phenomenon

Thayná Soares de Melo[1], Isadora de Oliveira Barbosa[1], Letícia Menegalli-Santos[1], Giovanna Ferranti de Castro[1], Aleksandra Trishina[2], Aldair Darlan Santos-de-Araújo[1], José Mário Prati[1], André Pontes-Silva[1*], Yury Zharikov[3]

A study published in the *Journal of the Brazilian Medical Association* examined the microglia role as the regulator of cognitive function[1]. Authors[1] reviewed what makes microglia so interesting to be studied as a possible therapeutic target in different conditions, starting with the analysis of its origins, passing through different conditions/diseases, and then discussing the possible future directions in research and clinic[1]. However, they[1] did not discuss the microglia role in the pain phenomenon as well as how it is related to cognitive function[2-4]. Therefore, as a contribution to the literature, we summarize the key findings on this topic (pain).

In neuropathic pain, microglia are morphologically and molecularly activated by opioids and contribute to opioid tolerance and dependence[5]. Activated microglia in the dorsal horn of the spinal cord are necessary for synaptic changes in this region and for pain hypersensitivity following nerve injury[6,7]. In addition, microglia are also activated in the brain and contribute to sensory and/or non-sensory (emotion, reward, and memory) aspects of neuropathic pain[5].

In chronic visceral pain, the mechanisms of microglia and astrocytes regarding the release of cytokines, chemokines, and neuroactive substances and the alteration of intracellular signaling pathways during the process are highlighted. As such, future perspectives include targeting microglia and astrocytes for chronic visceral pain treatment[8].

In chronic pain, microglia are indispensable for synaptic plasticity in the spinal dorsal horn and chronic pain[9]. Zhou et al.[9] showed that microglial colony-stimulating factor 1 and brain-derived neurotrophic factor signaling are essential for spinal long-term potentiation and chronic pain and that the microglia-dependent transition from synaptic potentiation to structural changes in pain pathways may underlie pain chronicity[9]. Besides, much scientific data suggest that classical activation of microglia in the spinal cord mediates neuroinflammation that plays an essential role in developing central sensitization and nociplastic pain[10].

In the early twentieth century, there was an increase in microglial studies in pain[11], with a particular focus on microgliosis in the spinal cord after nerve injury and in neuropathic pain[12]. We now know that signaling molecules are altered in microglia and contribute to the pathogenesis of pain; microglial mediators such as pro- and anti-inflammatory cytokines are potent neuromodulators that regulate synaptic transmission and pain via neuron-glial interactions; and microglia have an emerging role in pain resolution, in part via specialized pro-resolving mediators such as resolvins, protectins, and maresins[11].

Finally, pain, whether acute or chronic, involves inflammasome activation at the site of origin, the various relay stations, and the sensory and processing cortical areas[13]. Indeed, microglia are embedded in brain responses related to stress phenomena, the development of major depressive disorders (cognitive function), and pain-related neural processing[13]. However, there is a lack of robust or consistent clinical effects of microglial modulators due to the study designs and heterogeneity of the patient populations studied (or the underlying biology, of course)[11].

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[1]Universidade Federal de São Carlos, Physical Therapy Department – São Carlos (SP), Brazil.
[2]I.M. Sechenov First Moscow State Medical University (Sechenov University), Institute of Clinical Medicine – Moscow, Russia.
[3]I.M. Sechenov First Moscow State Medical University (Sechenov University), Department of Human Anatomy and Histology – Moscow, Russia.
*Corresponding author: contato.andrepsilva@gmail.com

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AUTHORS' CONTRIBUTIONS

TSM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **IOB:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **LMS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **GFC:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **AT:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **ADSA:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **JMP:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **APS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **YZ:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Lipedema: a new phenomenon for many people and a new field of study for psychiatry, nutrition, and psychology in Brazil

Jônatas de Oliveira[1*]

Brazil is one of the leading countries in plastic surgery and has cultural components that permeate body image, especially for women[1-4]. Despite less Brazilian data on eating disorders, body checking, dissatisfaction, and emotional pain support many women who seek procedures and treatments. Recently, much has been said about an unknown disease lipedema in many medical offices[5]. It was first described in the United States in 1940 and is characterized by fat accumulation in the lower regions[5]. There is a characteristic of disproportionality in the affected areas, and the symptoms appear throughout development, along with hormonal changes[6-8].

Recently, social networks have provided representations and connections between carriers of the disease, primarily women. These connections have allowed many of these individuals to recognize common symptoms, pain, and related suffering that are not normal. Many women have spent much of their lives with pain[9] and heaviness in their legs[10] due to fat accumulation, without knowing the name of this condition. It is classified as a disease of adipose tissue and is estimated to affect 11% of women[11]. It is often confused with lymphedema, another condition that can occur with lipedema.

Many physicians are still unaware of the condition, and patients may seek various treatments, such as nutrition or psychiatry, that correlate with the condition. Differential diagnosis requires understanding obesity, lipedema, and lymphedema, as well as the relationships among the three conditions[11]. Although almost no studies comment on the importance of body image in lipedema, it is essential to highlight that there is dissatisfaction with physical symptoms associated with various aspects such as beauty standards, body, and pressure to be thin which are already common in all individuals. Probably, individuals in conditions of socioeconomic exclusion and minorities should have a more significant impact on lipedema[12], and in Brazil, no studies are still carried out on this subject. Considering the double impact on carriers, one should consider studying body image in these patients. Is there full recognition of body shape? Is there distortion beyond what would be physically disproportionate? Is there body neglect in affected areas? Is there extreme control during treatments or diets? All of these questions need to be unraveled because they are associated with much suffering and can cause psychological and psychiatric distress and impairment[13]. Lipedema has been linked to emotional regulation difficulties and eating disorders[13,14], both of which are related to body image.

Recently, it has been suggested that a ketogenic diet may be beneficial and should be considered a treatment. This proposal comes from the Lipedema Project in Boston[11]. Many important questions need to be answered before this dietary intervention is considered, mainly the recruitment of cognitive restriction[15] necessary for low carbohydrate consumption and, in the case of eating disorders and disordered eating, the triggering in beliefs and cognitions of the overvaluation of body image, so well known in the treatment of eating disorders. In this case, considering lipedema, there is excess from the objective point of view, but from the subjective point of view, is it considered coherently? Or is there an internalized self-criticism and fatphobia in these patients? In my opinion, this population should be considered at high risk for disordered eating and eating disorders, and future research data should answer these questions. Future clinical challenges include how best to diagnose, treat, and manage this population[16] to improve their quality of life and health.

[1]Universidade de Sao Paulo, Faculty of Medicine, School of Medicine – Sao Paulo (SP), Brazil.
*Corresponding author: oliveira.jonatas@usp.br
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Postoperative atrial fibrillation and coronary bypass graft surgery: like two peas in a pod

Mesut Engin[1*], Nurcan Kacmaz Kat[2]

Dear Editor,

We have read the article entitled "Inflammatory prognostic index predicts new-onset atrial fibrillation and mortality after on-pump coronary artery bypass grafting" by Badem et al.[1] with great interest. First of all, we congratulate the authors for their valuable contribution to the literature. However, we would like to discuss some points about postoperative atrial fibrillation after coronary artery bypass graft (CABG) surgery.

First of all, we think that the diagnosis of PoAF in the study should be clarified. The study was planned retrospectively. Also, the authors stated that they performed rhythm monitoring from the day of operation until discharge. Do you routinely follow the continuous rhythm in patients undergoing CABG in your clinic? Are you using continuous wearable telemetry monitoring on all patients? It should be stated more clearly how rhythm monitoring is done during the intensive care unit and service. In addition, the authors defined PoAF as atrial fibrillation (AF) rhythm lasting longer than 10 min or an unstable hemodynamic condition. Why did you set the duration as 10 min? According to many studies in this literature, it has been determined as 30 min or 60 s[2]. Therefore, I would like to ask this question as well. Among the patients who did not develop PoAF, how many patients developed an attack of AF between 5 and 9.9 min?

Various inflammatory values obtained from routine blood parameters are widely used in the diagnosis of cardiovascular diseases and prediction of prognosis. Among these, C-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR), parameters related to platelet, and albumin values are found to be important[3-5]. In their study, the authors revealed that the inflammatory prognostic index (CRPxNLR/Albumin) value obtained from the preoperative blood values of the patients may be a predictor for PoAF[1]. Also, all patients underwent coronary artery bypass graft (CABG) surgery accompanied by cardiopulmonary bypass (CPB). The effects of CPB systems on blood parameters are known[6]. As a result, each patient's CRP, NLR, and albumin values will change after CPB. We think that the fact that postoperative blood values were not included in the study should be stated as an important limiting point.

In addition, CPB systems can be used in different forms today. Were standard CPB systems used in all patients? Were the pump line lengths the same in the patients? Is albumin added to prime solutions in all patients? How did you achieve initial and maintenance diastolic arrest in your patient group? As is known, different initial cardioplegia solutions may affect the PoAF rates and other clinical results[7,8]. Recently, minimally invasive extracorporeal circulation (MiECC) circuits have also been used in CABG operations[9]. Was MiECC used in your patient group? Since these situations may affect PoAF development rates, clarification of these points will increase the value of the study.

Finally, we would like to mention some points about the multivariate logistic regression analysis. In the study, a multivariate analysis model was created based on the values that were significant in univariate analysis. So why was the aspartate aminotransferase value, which was not significant in univariate analysis, included in the multivariate model? Apart from that, why were not variables such as reintubation, low cardiac output, and infection, which could affect the development of PoAF and were significant in univariate analysis, not included in the multivariate model? The use of inotropic agents due to low cardiac output and respiratory infections may affect the development of PoAF[10,11].

AUTHORS' CONTRIBUTIONS

ME: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **NKK:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

[1]University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Department of Cardiovascular Surgery – Bursa, Turkey.
[2]University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Department of Radiology – Bursa, Turkey.
*Corresponding author: mesut_kvc_cor@hotmail.com

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Comment on "Evaluation of platelet indices and pro-inflammatory cytokines in type 2 diabetic patients with retinopathy"

Yujie Hou[1], Junlin Qiu[2*]

Dear Editor,

We read with great interest the recent study by Kucuk et al.[1], which investigated the relationship between platelet indices, pro-inflammatory cytokines, and the presence of retinopathy in individuals diagnosed with type 2 diabetes. This research offers valuable insights into the complex interplay between diabetes, retinopathy, and inflammatory markers. The study's meticulous analysis of platelet indices revealed a significant correlation between these indices and the severity of diabetic retinopathy. This discovery holds promise in enhancing our understanding of the underlying mechanisms contributing to retinopathy's progression. Furthermore, the inclusion of pro-inflammatory cytokines in the investigation adds points to the discussion, suggesting a potential link between inflammation and retinopathy severity. This study underscores the importance of considering platelet indices and pro-inflammatory cytokines in assessing and managing diabetic retinopathy. The findings have the potential to guide future research and therapeutic approaches aimed at mitigating the impact of this condition. Nevertheless, certain concerns among the following warrant additional clarification.

First, as described in Table 1 of Kucuk's study[1], it is evident that the type 2 diabetic patients' ages were markedly higher in comparison to the healthy controls (54.18±9.61 versus 50.04±8.93, p=0.012). This discrepancy hints at a significant age distinction between the two groups. Interestingly, a study by Cetin et al[2]. indicated a moderate negative correlation between plateletcrit (PCT) and age (r=-0.330), implying that an increase in age might correspond to a decrease in the PCT levels. In light of this finding, it is intriguing to consider the results of the study conducted by Kucuk et al., which revealed that type 2 diabetic patients had lower PTC levels compared to healthy controls (0.20±0.06 versus 0.23±0.04, p<0.001). This discrepancy in PTC levels between the two groups may not be attributed to diabetes itself, but rather potentially influenced by the higher average age among the type 2 diabetic patients. Similarly, the variations observed between type 2 diabetic patients and healthy controls in terms of mean platelet volume (MPV) and interleukin-1alpha (IL-1α) expression were not likely due to diabetes but due to the age differences between the two cohorts. Consequently, it becomes imperative to adequately adjust for age, a confounding factor, prior to delving into the data analysis.

Second, upon examining the data presented in Table 1 of the study, a particular observation arises. The average HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) for type 2 diabetic patients is noted as 5.55, accompanied by a corresponding standard deviation of 6.37. This strikingly reveals that the value of the standard deviation is notably greater than the mean (6.37 compared to 5.55). Such a scenario strongly suggests that HOMA-IR data might possess a skewed distribution, thereby warranting the use of the Wilcoxon rank sum test for intergroup comparisons rather than the Student's t-test. Similarly, this can be applied to the parameter p-selectin. Among type 2 diabetic patients, the mean p-selectin value is reported as 15.78, alongside a standard deviation of 11.05. Conversely, among healthy controls, the mean p-selectin value is 13.76, accompanied by a standard deviation of 13.51. Thus, to ensure the accuracy of conclusions drawn, it is crucial to employ appropriate statistical methods that account for the potential skewed distribution of data.

AUTHORS' CONTRIBUTIONS

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[1]Central South University, Guilin Hospital of the Second Xiangya Hospital, Department of Endocrinology – Guilin, China.
[2]Central South University, Graduate School of the Second School of Clinical Medicine, Department of Endocrinology and Metabolism – Changsha, China.
*Corresponding author: Qiujunlin2003@163.com

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Comment on "Importance of epicardial adipose tissue as a predictor of heart failure with preserved ejection fraction"

Xianfeng Qin[1], Chan Yu[2*]

Dear Editor,

We read the recent study performed by Ateş et al[1]. with great interest, which underscores the importance of epicardial adipose tissue as a predictor for heart failure with preserved ejection fraction. The research highlights the pivotal role of this adipose tissue in anticipating the likelihood of developing this specific type of heart failure. Through an exploration of its correlation with various clinical parameters and outcomes, the study emphasizes the potential utility of epicardial adipose tissue as a valuable prognostic marker for individuals at risk of heart failure with preserved ejection fraction. These findings provide insightful perspectives into the complex interplay between adipose tissue and cardiovascular health, thereby opening avenues for more precise risk assessment and targeted interventions in clinical practice. Nevertheless, there are certain issues that warrant attention and resolution.

First, there is some ambiguity regarding the variables included in the univariate and multivariable logistic regression analyses in this study[1]. As indicated in Table 2 of the study[1], variables such as age, epicardial adipose tissue (EAT), left ventricular mass index (LVMI), waist circumference, left atrial volume index (LAVI), and E/é were incorporated into both univariate and multivariable logistic regression analyses. However, it remains unclear whether other factors like hemoglobin, NT-proBNP (N-terminal pro-brain natriuretic peptide), and glomerular filtration rate (GFR) were also included in the logistic regression analysis. Previous studies have underscored the relevance of various factors to heart failure prognosis, notably NT-proBNP. For instance, a study[2] encompassing 3,562 heart failure patients with preserved ejection fraction demonstrated a correlation between elevated NT-proBNP levels and baseline characteristics, including atrial fibrillation, NYHA IV symptoms, and lower estimated glomerular filtration rate. Yet, Table 2 of the present study[1] does not depict the relationship between NT-proBNP and heart failure with preserved ejection fraction. We are wondering whether all confounding factors, such as NT-proBNP, were included in the univariate and multivariable logistic regression or only a subset of the parameters from Table 1[1] was considered. Given these points, a more comprehensive understanding of the factors considered in the multivariable analysis would greatly enhance the study's findings. It would also aid in evaluating the potential interactions and interplay of various variables in predicting heart failure with preserved ejection fraction.

Furthermore, as described in the study[1], epicardial adipose tissue was assessed using transthoracic echocardiography. However, the study does not provide a detailed account of the timing of the echocardiographic examinations – were they conducted prior to medication treatment, during treatment, or post-treatment? Considering that this timing significantly correlates with epicardial adipose tissue measurements, clarity on this aspect would provide valuable context for interpreting the results accurately. Therefore, it becomes evident that understanding the relationship between the timing of echocardiographic assessments and epicardial adipose tissue measurements could offer deeper insights into the dynamics of this tissue in the context of heart failure with preserved ejection fraction. Addressing this temporal aspect would undoubtedly enhance the comprehensiveness of the study's findings and their potential implications for clinical practice.

AUTHORS' CONTRIBUTIONS

XQ: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **CY:** Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing.

[1]Guangzhou University of Chinese Medicine, First Clinical Medical School – Guangzhou, China.
[2]The First Affiliated Hospital of Guangdong Pharmaceutical University, Department of Cardiology – Guangzhou, China.
*Corresponding author: yc_cheryl@163.com

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Early-term prognosis in patients with acute aortic dissection: calm before the storm

Mesut Engin[1*], Ahmet Burak Tatlı[1]

Dear Editor,

We have read the article by Dönmez et al.[1], entitled "Evaluation of descriptive performances of platelet indices, neutrophil/lymphocyte ratio, and platelet/lymphocyte ratio in aortic dissections" with great interest. First of all, we congratulate the authors for their valuable contribution to the literature. However, we would like to discuss some points about acute aortic dissection and its early-term mortality.

Acute aortic dissections are important cardiovascular emergencies with high perioperative mortality and morbidity which require urgent interventions[2]. Various parameters obtained from routine blood values have been investigated in the literature to predict early clinical outcomes. These are usually neutrophil, lymphocyte, C-reactive protein, and platelet-related parameters[3,4]. However, while investigating these parameters as clinical predictors, we think that important determinants of mortality should be included in the analysis. The most important of these determinants are the types of performed intervention according to dissection types (surgery, endovascular intervention, medical treatment, etc.), the perfusion methods used in the surgery, the amount of blood products used, the preoperative left ventricular ejection fraction, the presence of low-cardiac output syndrome, and the time between surgery and the first symptom[5]. In addition, in the case of type 2 dissection, only the ascending aorta limited dissection status may also have an impact on mortality[6].

However, the rate of DeBakey Type 1 dissection in the 30-day survival group in the study was approximately twice that of the 30-day mortality group. The DeBakey Type 2 dissection rate was more than twice in the 30-day mortality group than in the 30-day survival group. This information is quite different from the known literature. What do you think is the reason for this situation? Were the Type 2 dissection patients in the study not operated? Did the patients not accept the operation? Was there a preoperative cerebrovascular accident or heart failure? Did it consist of patients who had had previous cardiac surgery? Otherwise, it is clear that Type 1 dissection is a high mortal condition in the early- and long-term period[7].

Various cerebral protection methods can be used in Type 1 aortic dissection (61.3% of patients in your study) surgery, including unilateral/bilateral antegrade cerebral perfusion, retrograde cerebral perfusion, and deep hypothermia. The effect of this situation on morbidity has been demonstrated[8]. Early clinical results may be affected surgically, depending on the surgical area in the aorta[9].

As a result, it may mislead us to reach a conclusion on the early mortality of acute aortic dissections only from the blood values at the time of admission. The patients are open to many treatments from the moment they are admitted to the emergency department. Of course, admission blood values may affect mortality, but it would be useful to add data from interventional procedures to multivariate analyses investigating the causes of mortality.

AUTHORS' CONTRIBUTIONS

ME: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.
ABT: Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

[1]University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Department of Cardiovascular Surgery – Bursa, Turkey.
*Corresponding author: mesut_kvc_cor@hotmail.com

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ORIGINAL ARTICLE

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Different methods for assessing glomerular filtration rate in the elderly

Gilsirene Scantelbury de Almeida[1*], Noeli das Neves Toledo[1], Miharu Maguinoria Matsuura Matos[2], Luis Cuadrado Martin[3], Roberto Jorge da Silva Franco[3]

SUMMARY

OBJECTIVE: The objective of this study was to identify the best method to replace cystatin C in the evaluation of glomerular filtration in the elderly.

METHODS: Individuals over 60 years of age from a primary care center were studied. Blood was collected to determine creatinine and cystatin C and 24-h urine. Three methods were compared to determine glomerular filtration: Creatinine clearance, Cocroft-Gault, modification of diet in renal disease, and Collaboration Epidemiology of Chronic Kidney Disease based on creatinine, considering as a reference the determination of glomerular filtration using the cystatin-based Chronic Kidney Disease Epidemiology Collaboration equation. The statistical methods used were linear regression, Bland-Altman curve, and receiver operating characteristic.

RESULTS: A total of 180 elderly people were evaluated, but 14 patients were lost from the sample, resulting in a total of 166 patients. The average age of patients was 66.9±6.1 years, and 69.8% were females. Regarding the number of patients eligible for the study, there were 12 black, 108 brown, and 46 white, 42.77% hypertensive, and 38.3% diabetic. Glomerular filtration was less than 60 mL/min in 22.28% of patients. Regarding the evaluation of the different equations, the correlation coefficient was lower for creatinine clearance and progressively higher for Cocroft-Gault, modification of diet in renal disease, and Collaboration Epidemiology of Chronic Kidney Disease based on creatinine. The Bland-Altman diagram and the receiver operating characteristic curve showed similar performance to the correlation coefficient for the different equations evaluated.

CONCLUSION: Collaboration Epidemiology of Chronic Kidney Disease based on creatinine presented the best performance. Creatinine debug had the worst performance, which reinforces the idea that 24-h urine collection is unnecessary in these patients.

KEYWORDS: Glomerular filtration rate. Cystatin C. Chronic kidney disease.

INTRODUCTION

In Brazil, it is estimated that about 20 million people have chronic kidney disease (CKD), which is strongly associated with morbidity and mortality[1]. The diagnosis of CKD should be based on the persistence, for 3 months or more, of a glomerular filtration rate (GFR) £60 mL/min or on the structural or functional abnormality of the kidney, which is demonstrated by pathological changes or by markers of renal injury, even if abnormalities are present as assessed by blood, urine, or imaging tests. A chronically low GFR (<60 mL/min/1.73 m^2) is sufficient to make the diagnosis of CKD, with or without other markers of kidney damage[2].

The cystatin-based Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI-Cyst) is based on the creatinine and cystatin C levels. The lysosomal protein cysteine is particularly attractive as a marker of kidney function[3]. The use of cystatin C may be particularly advantageous in elderly patients, as it is the method with the best validation in this age group[4]. However, the dosage of this substance is still unfeasible in most centers due to its high price and the lack of availability of this test in the Unified Health System. This fact leads to the search for alternatives to cystatin C. Evaluating whether GFR estimation equations based only on creatinine could be used instead of the equation that adopts cystatin C and verifying whether these equations can estimate the GFR obtained by cystatin C are of practical interest, especially in places with limited financial resources.

Therefore, the aim of this research was to identify the best method to replace cystatin C in the assessment of GFR in the elderly. Equations based on serum creatinine levels were compared, and the equation based on cystatin C were adopted as reference.

[1]Universidade Federal do Amazonas, Manaus School of Nursing – Manaus (AM), Brazil.
[2]Universidade Federal do Amazonas, Getúlio Vargas University Hospital – Manaus (AM), Brazil.
[3]Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculty of Medicine – Botucatu (SP), Brazil.
*Corresponding author: gilscantelbury@ufam.edu.br
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METHODS

This is a cross-sectional study to verify the diagnostic performance of different methods of glomerular filtration assessment in an elderly population sample. Four methods were compared, namely, Cockroft-Gault (C&G), modification of diet in renal disease (MDRD), Collaboration Epidemiology of Chronic Kidney Disease based on creatinine (CKD-EPI-Cr), and creatinine release (Cr). GFR assessed by CKD-EPI-Cyst was adopted as the standard. Although inulin is considered the ideal marker of GFR since it is freely filtered by the glomerulus and is not reabsorbed or secreted by the renal tubule, direct measurement of GFR using inulin is not feasible because it requires continuous intravenous infusion and a fixed time for urine collection. Due to age-related limitations, the high prevalence of prostatic disease in the elderly leads to inaccuracies in 24-h urine volume and the possible presence of residual volume. We adopted CKD-EPI-Cyst as the standard of analysis.

Data collection was carried out at the Center for Integrated Care for the Elderly (CAIMI), a service center created to provide care exclusively to individuals aged 60 years and above. CAIMI is an interdisciplinary care service center that involves in activities in the areas of social work, nursing, pharmaceutics, laboratory, and medical assistance. They are distributed throughout the city of Manaus-AM in the health districts located in all directions. The CAIMI located in the western district was chosen to conduct the research because it is the most representative of the target population. Patients aged above 60 years who sought general clinical care for the first time were included. The age group chosen was due to the orientation made by the National Health Policy of the Elderly Person in Brazil, in line with the principles and guidelines of the Unified Health System. These consider Brazilians aged 60 years or older to be elderly[5]. Patients who sought health care at this CAIMI from September to November 2012 were included sequentially. Patients from other specialties with suspected kidney problems and older adults who were unable to answer the questionnaire due to cognitive limitations and unaccompanied were excluded. Creatinine and cystatin levels were measured in a single blood sample.

Weight and height measurements were carried out using an electronic scale and a portable stadiometer, respectively. The elderly were weighed standing with barefoot and wearing light clothing. The tests were collected in the morning with fasting for 12 h. Blood samples were stored at -80°C. A 24-h urine collection was performed the day before the blood test. The tests were performed using commercial kits from Winner on an automated BT 3000 plus equipment from Winner Lab, Rome, Italy. Samples were processed for serum and urine creatinine and serum cystatin C assay. Cystatin C was measured by immunoturbidimetry. The equations used in the study were as follows:

C&G equation

GFR=[(140–age in years)×Weight (kg)/Cr (mg/dL)×72]×0.85 (female) or 1.0 (male) (mL/min)[6].

The value was adjusted for body surface area using the Dubois & Dubois formula (mL/min/1.73 m^2)[7].

MDRD equation

GFR=186×[Cr (in mg/dL)]–1.154×[age (years)]–0.203×[0.742 (if female)×[1.212 (if black)] (mL/min/1.73 m^2)[8].

CKD-EPI-Cr 2009 equation[9]

Correcting for sex of serum creatinine (mg/dL) in case of female ≤0.7, the estimated GFR equation is 144×(Creat/0.7)–0.329×0.993 years [×1159 if black] or ≥0.7 144×(Creat/0.7)–1209×0.993 age [×1159 if black]. In men ≤0.9, the estimated GFR equation is 144×(Creat/0.9)–0.401×0.993 age [×159 if black] or ≥0.9 144×(Creat/0.9)–1.209×0.993 age [×159 if black].

CKD-EPI-Cyst 2012 equation[9]

Correcting for sex of serum creatinine (mg/dL) being female ≤0.7 and cystatin C (mg/L) ≤0.8, the estimated GFR equation is 130×(Creat/0.7)–0.248×(Cys/0.8)–0.375×0.995 age [××1.08 if black] or if ≤0.7 and cystatin C (mg/L)>0.8, the estimated GFR equation is 130×(Creat/0.7)–0.248×(Cys/0.8)–0.711×0.995 [×1.08 if black]. However, if serum creatinine (mg/dL)>0.7 and cystatin C (mg/L)≤0.8 130×(Creat/0.7)–0.601×(Cys/0.8)–0.375×0.995 age [×1.008 if black] or if serum creatinine (mg/dL)>0.7 and cystatin C (mg/L)>0.8130×(Creat/0.7)–0.601×(Cys/0.8)–0.711×0.995 age [×1.08 if black].

In men, creatinine (mg/dL) ≤0.9 and cystatin C (mg/L) 0.8, the estimated GFR equation is 135×(Creat/0.9)–0.207×(Cys/0.8)–0.375×0.995 age [×01.08 if black] or if ≤0.9 and cystatin C (mg/L)>0.8 135×(Creat/0.9)–0.207×(Cys/0.8)–0.711×0.995 age [×1.08 if black]. However, if creatinine (mg/dL)>0.9 and cystatin C (mg/L), the estimated glomerular filtration rate equation is 135 × (Creat/0.9)≤0.8–0.601×(Cys/0.8)–0.375×0.995age [×1.08 if black] or if Creatinine (mg/dL)>0.9 and Cystatin C (mg/L)>0.8135×(Creat/0.9)–0.601×(Cys/0.8)–0.711×0.995 age [×1.08 if black].

Statistical analysis

The sample consisted of 180 elderly people, sufficient to detect a correlation coefficient of 0.25 with a beta error of 0.2 and an alpha error of 0.05. Data were presented as mean±standard deviation. Qualitative variables were described as absolute frequency and percentage. Glomerular filtration values by the different methods were compared with the standard (CKD-EPI-Cyst). To compare the methods, scatterplots were constructed, and the correlation coefficient, as well as Bland

Altman diagrams and receiver operating characteristic (ROC) curves, was calculated. For statistical inferences, the significance level ($p<0.05$) was considered. Statistical analyses were performed using SPSS 21.0. This research was approved by the Research Ethics Committee of the Federal University of Amazonas, under number 0261.0115.000-10.

RESULTS

A total of 180 elderly people were examined. However, there was a sample loss of 14 individuals, and of the 166 eligible, there were no patients with clinically manifest cancer in this sample. Socio-demographic and clinical data are described in Table 1.

Evaluation of the Cockcroft-Gault equation

The comparison of the determination of glomerular filtration by the Cockcroft-Gault formula with glomerular filtration determined by the CKD-EPI-Cyst equation is presented in Figure 1. Pearson's correlation coefficient was 0.515 ($p<0.001$), and r^2 was 0.254.

Evaluation of the modification of diet in renal disease equation

The comparison of the determination of glomerular filtration by the MDRD equation with glomerular filtration determined by the CKD-EPI-Cyst equation is shown in Figure 2. A Pearson correlation coefficient of 0.568 ($p<0.001$) and an r^2 of 0.327 were observed.

Table 1. Socio-demographic and clinical data of the elderly at the Center for Integrated Care for the Elderly in Manaus/Amazonas/Brazil.

Variables	Number	%
Age	66.9 (6.1)	
Sex		
Men	50	30.1
Women	116	69.8
Race and color		
White	46	27.71
Black	12	7.22
Brown	108	65.07
Hypertensive	71	42.77
Women	50	30.12
Men	21	12.65
Kidney disease	34	20.48
Diabetes	69	38.3
Body mass index	28.07 (4.7)	27.67 (18.81–46.34)
Low weight	17	10.24
Suitable weight	60	36.14
High weight	103	62.04
Medicines used		
Antihypertensive	93	56.02
Antiglycemic	33	19.88
Did not use medications	40	24.10
Glomerular filtration rate		
Internship I (TFGe>90)	33	19.88
Internship II (TFGe between 90 and 60)	96	57.84
Internship IIIa (TFGe between 60 and 45)	24	14.45
Internship IIIb (between 45 and 30)	13	7.83
Internship IV (between 30 and 15)	0	0
Internship V (<15)	0	0

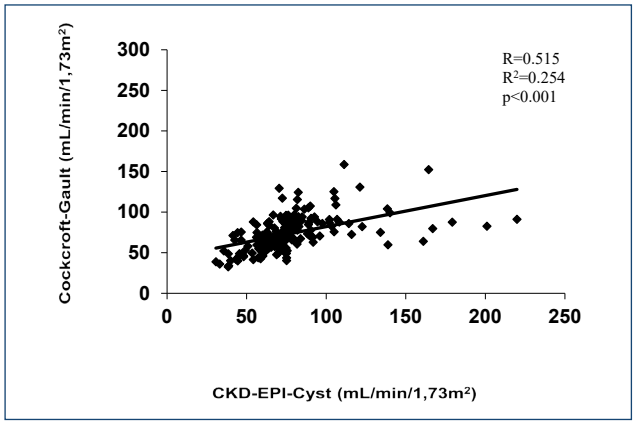

Figure 1. Scatter diagrams, Bland-Altman, and receiver operating characteristic curves for the comparison of Cockroft-Gault with the reference of the equation of cystatin-based Chronic Kidney Disease Epidemiology Collaboration.

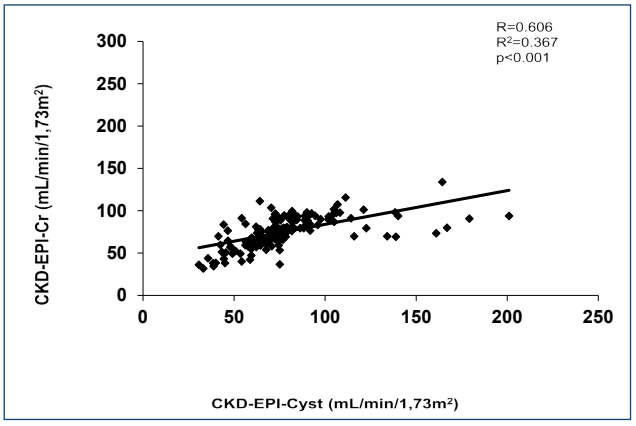

Figure 3. Scatter diagrams, Bland-Altman, and receiver operating characteristic curves for the comparison Collaboration Epidemiology of Chronic Kidney Disease based on creatinine from CKD-EPI-Cys with the reference of the equation of cystatin-based Chronic Kidney Disease Epidemiology Collaboration.

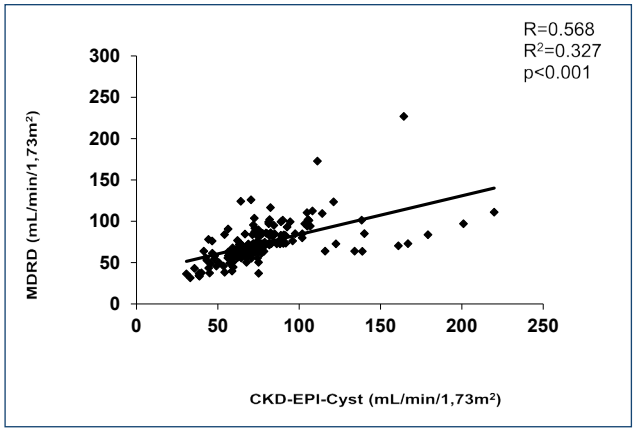

Figure 2. Scatter diagrams, Bland-Altman, and receiver operating characteristic curves for the comparison of modification of diet in renal disease from Collaboration Epidemiology of Chronic Kidney Disease based on creatinine with the reference of the equation of cystatin-based Chronic Kidney Disease Epidemiology Collaboration.

Evaluation of the Collaboration Epidemiology of Chronic Kidney Disease based on creatinine equation

The comparison of glomerular filtration determined by the CKD-EPI-Cr equation with glomerular filtration determined by the CKD-EPI-Cyst equation is shown in Figure 3. A Pearson correlation coefficient of 0.606 ($p<0.001$) (Figure 1) and an r^2 of 0.367 were observed.

DISCUSSION

Chronic kidney disease is a worldwide public health problem that mainly affects the elderly. The utilization of creatinine dosage equations has become the most common method for evaluating glomerular filtration[10]. Thus, this study aimed to identify the best method to replace cystatin C in the evaluation of glomerular filtration in the elderly. It was observed that the GFR evaluated by the CKD-EPI-Cr formula was the one that was closest to the adopted standard.

Creatinine is the endogenous marker most commonly used in clinical practice, either by applying equations based on its serum determination or in conjunction with 24-h urine collection for CrCl determination. The latter method also has several disadvantages. These include the difficulty of collecting 24-h urine[11].

The National Kidney Foundation guideline recommends "not using creatinine alone as a method of assessing the level of renal function." A practical clinical solution to the problem is to use creatinine to estimate GFR using equations that include parameters correlated with muscle mass, namely, age, gender, ethnicity, and body weight[11]. Several equations, such as CocG, MDRD, CKD-EPI-Cr[12], and CKD-EPI-Cyst[13], endorsed by the National Kidney Foundation have been proposed. The CocG equations, like the MDRD, underestimate GFR in populations with high GFR levels, such as type 1 diabetics without microalbuminuria and kidney transplant donors[14]. In this study, they are in line with the literature, as can be seen in the Bland-Altman diagram. These equations have not been validated for children, older adults over 70 years, pregnant women, or other demographic subgroups. Studies using the established gold standard have concluded that the MDRD equation appears to be more accurate than the CocG equation[15]. We identified this finding in the study; therefore, the performance assessment parameters of MDRD were better than CocG when compared with CKD-EPI-Cyst.

The MDRD equation is thought to underestimate GFR in individuals with normal or increased GFR, but as is clear from

the Bland-Altman diagram in this study, there was no association between GFR level and deviations from the standard used. The equation that considers race in the calculation of glomerular filtration was used, since at the time of this study, however, the CKD-epi equation that disregards this demographic variable had not been developed.

The definition of race in the Brazilian population is made difficult by the high degree of miscegenation, but this limitation of our study tends to underestimate our findings and increase the dispersions observed. Therefore, if we could define the race of our patients absolutely, our results would be better. Thus, despite the uncertainty regarding the definition of race, we obtained positive data.

In the regression between GFR assessed by CKD-EPI-Cyst and GFR assessed by ClCr, we observed that there is a statistically significant correlation. However, there is a marked dispersion of the points. When CKD-EPI-Cyst was zero, ClCr was 62.1 mL/min/1.73 m^2, which characterizes an overestimation of the real GFR value, making this method of GFR assessment unfeasible in this population. Our results support the idea that the usefulness of GFR using CrCl with 24-h urine collection should be re-evaluated in medical practice.

Validation of the CocG equation was based on hospitalized men aged 18–92 years with normal renal function as the basis for estimating creatinine clearance. It was not standardized to a body surface area of 1.73 m^2 and was corrected for women. It underestimates GRF because tubular creatinine secretion and weight gain due to obesity or fluid overload are not taken into account[16]. These observations are consistent with the data in this study.

This study had some merits to be highlighted. It was carried out exclusively with elderly people, a group that has been less evaluated in studies that developed various equations for calculating the GRF estimate. Another merit of the study was the selection of primary care patients, which simulates the condition that represents real life in general outpatient clinics.

In this investigation, the percentage of study patients who had glomerular filtration lower than 60 mL/min was 22.28%, which is equivalent to the national prevalence of CKD in a study in Brazil, which is 21.4% in the elderly[2]. This was not a randomized study.

CONCLUSION

Creatinine clearance and the C&G equation showed poor performance in predicting GFR estimated by cystatin-C. Both the MDRD study GFR calculation equation and CKD-EPI-Cr can be used as substitutes when cystatin-C dosage is not available, with a slight advantage for CKD-EPI-Cr.

AUTHORS' CONTRIBUTIONS

GSA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **NNT:** Investigation, Methodology. MMMM: Investigation, Methodology. **LCM:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **RJSF:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Prevalence of early neonatal sepsis and positive maternal culture for group B beta-hemolytic *Streptococcus*

Cecília Gomes Cunha Silva[1], Maria Júlia Arantes Leobas[1], Andressa Paes Medeiros de Freitas[1], Júlia Teoro Mansano[1], Jaider Antonio Vidigal Rodrigues[1], Edward Araujo Júnior[2,3*], Alberto Borges Peixoto[1,4]

SUMMARY

OBJECTIVE: The aim of this study was to evaluate the prevalence of early neonatal sepsis in pregnant women with a positive culture for group B beta-hemolytic *Streptococcus* in a middle-income city in Southeastern Brazil.

METHODS: A retrospective cohort study was conducted, involving singleton low- and high-risk pregnancies in whom group B beta-hemolytic *Streptococcus* cultures were evaluated between 35 and 37 weeks of gestation using vaginal and anal swabs. A specific medium (Todd-Hewitt) was used for culturing. The pregnant women were divided into two groups based on positive (n==201) and negative (n==420) cultures for group B beta-hemolytic *Streptococcus*.

RESULTS: The maternal colonization rate by group B beta-hemolytic *Streptococcus* was 32.3%. The prevalence of early neonatal sepsis was 1.0% (2/201) among patients with a positive group B beta-hemolytic *Streptococcus* culture and 1.9% (8/420) among patients with a negative culture. Among the patients who underwent adequate prophylaxis, crystalline penicillin G was used in 51.9% (54/104), followed by cefazolin in 43.3% (45/104), ampicillin in 3.8% (4/104), and clindamycin in 1.0% (1/104). A model that included prematurity (p==0.001) proved to be an independent risk predictor of early neonatal sepsis [χ^2 (1)==15.0, odds ratio: 16.9, 95% confidence interval: 4.7–61.6, p<0.001, Nagelkerke R^2==0.157].

CONCLUSION: The prevalence of a positive culture for group B beta-hemolytic *Streptococcus* was high. However, the prevalence of early neonatal sepsis was low in pregnant women with both positive and negative group B beta-hemolytic *Streptococcus* cultures and in pregnant women with a positive culture who underwent both adequate and inadequate antibiotic prophylaxis. Prematurity proved to be an independent predictor of early neonatal sepsis, considering the entire study population.

KEYWORDS: *Streptococcus*. Neonatal sepsis. Antibiotic prophylaxis. Pregnancy outcomes.

INTRODUCTION

Group B beta-hemolytic *Streptococcus* (GBS) is an important cause of maternal and neonatal morbidity and mortality. In pregnant women, maternal colonization by GBS is associated with adverse perinatal outcomes, such as low birth weight, preterm birth, and premature rupture of ovular membranes (PROM)[1]. In newborns, GBS is an important cause of neonatal sepsis, meningitis, and pneumonia[2]. Neonatal infection by GBS is divided into early (within the first week of life) and late (between 1 week and 3 months of life) onsets[3], and maternal colonization by GBS is the leading cause of early neonatal sepsis in newborns[4].

The rate of maternal GBS colonization was observed to be 13.4% in a study conducted in Saudi Arabia[5] and 14.6% in a study conducted in Ethiopia[6]. Therefore, universal screening using vaginal and anal swabs between 35 and 37 weeks of gestation is recommended for GBS detection. The primary risk factor for neonatal GBS early-onset disease is maternal colonization of the genitourinary and gastrointestinal tracts. Vertical transmission usually occurs during labor or after the rupture of membranes[1,7].

Intrapartum administration of antibiotics reduces the rate of early neonatal sepsis due to GBS, with crystalline penicillin G being the most commonly used antibiotic[8]; however, high resistance rates have been described for antibiotics such as clindamycin and erythromycin[9]. Inadequate antibiotic prophylaxis for maternal colonization by GBS may lead to increased rates of early neonatal sepsis[10].

Prematurity, low birth weight, and antepartum fetal tachycardia proved to be significant risk factors for pneumonia and sepsis, whereas prematurity, low birth weight, and an anomalous presentation were identified as risk predictors for neonatal

[1]Universidade de Uberaba, Mário Palmério University Hospital, Gynecology and Obstetrics Service – Uberaba (MG), Brazil.
[2]Universidade Federal de São Paulo, Escola Paulista de Medicina, Department of Obstetrics – São Paulo (SP), Brazil.
[3]Universidade Municipal de São Caetano do Sul, Medical Course – São Caetano do Sul (SP), Brazil.
[4]Universidade Federal do Triângulo Mineiro, Department of Gynecology and Obstetrics – Uberaba (MG), Brazil.
*Corresponding author: araujojred@terra.com.br
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conjunctivitis. Positive GBS cultures were found in 46% of neonatal sepsis cases[11].

The main objective of this study was to evaluate the prevalence of early neonatal sepsis in pregnant women with a positive culture for GBS in a middle-income city in Southeastern Brazil. Secondary objectives were to evaluate the association between positive and negative cultures for GBS, PROM, and preterm birth and to evaluate the best predictors of early neonatal sepsis in patients who underwent culture for GBS.

METHODS

A retrospective cohort study was conducted at the Mário Palmério University Hospital (MPHU) in the city of Uberaba, Minas Gerais, Brazil, by analyzing the medical records of pregnant women who attended the hospital from March 2016 to March 2019. According to GBS culture, pregnant women included were separated into two groups: positive GBS culture and negative GBS culture. Subsequently, for secondary analyses, pregnant women with positive culture were subdivided into adequate prophylaxis and inadequate prophylaxis for GBS. The study was approved by the Research Ethics Committee of the University of Uberaba (CAAE: 52299421.7.0000.5145).

The study included all patients with singleton pregnancies and without fetal malformations or chromosomal anomalies who were treated in the MPHU's low- and high-risk prenatal outpatient clinics, delivered in the MPHU's labor ward, and had a GBS culture performed between 35 and 37 weeks of gestation.

In our service, pregnant women are routinely screened for GBS cultures after vaginal and perianal swab collection. Immediately after collection, each swab was individually inserted into a tube containing Stuart transport medium (Biocon®, Belo Horizonte, Brazil) and stored at room temperature before being sent to the laboratory within a maximum period of 3 days.

In the laboratory, the material was cultured in a specific medium (Todd-Hewitt), which provides essential nutrients for the development of the microorganism while partially inhibiting other microorganisms. At a temperature of 35°C–37°C, the reading was taken manually to identify the growth and count of GBS colonies after 24 h in this environment.

In our service, prophylactic antibiotics are indicated for all pregnant women with a positive culture for GBS upon admission for induction of labor or labor management, except for those undergoing cesarean section with intact membranes (prophylactic antibiotics are administered prior to skin incision). According to the institutional protocol, prophylactic antibiotics are administered to all pregnant women with a negative culture taken 5 weeks or more ago in labor with rupture of membranes lasting more than 18 h. If crystalline penicillin is unavailable, a 2-g intravenous (i.v.) dose of ampicillin (i.v.) is given as a loading dose, followed by 1 g (i.v.) every 6 h until delivery. In case of penicillin allergy, clindamycin 900 mg (i.v.) is given every 8 h until delivery. In patients undergoing cesarean section, cefazolin 2 g (i.v.) may also be given as a loading dose, followed by 1 g (i.v.) every 6 h until delivery. Administration of two doses of any antibiotic within 4 h of delivery is considered adequate prophylaxis[12].

The following outcomes were considered adverse perinatal outcomes: neonatal sepsis, maternal admission to the intensive care unit (ICU), chorioamnionitis, neonatal ICU admission, Apgar score<7 at 1st minute, and early neonatal death (up to 48 h of life). Maternal and infant ICU admission was based on any clinical instability that warranted intensive care. Not all cases admitted to the ICU as a result of GBS infection were included in the study.

The presence of any of the following abnormalities was considered early neonatal sepsis: leukocytes <5,000/mm^3 or >25,000/mm^3 at birth, >30,000/mm^3 at 12–24 h of life, or ≥21,000/mm^3 at 2 days of life; increased immature neutrophils; platelets<150,000/mm^3; immature/total neutrophil ratio>0.3; and the presence of signs such as lethargy, irritability, thin pulse, cyanotic extremities, and tachypnea[13].

Quantitative variables were subjected to a normality test (D'Agostino-Pearson). The mean and standard deviation were used to represent variables with a normal distribution. The median and interquartile range were used to represent variables with a non-normal distribution. Categorical variables were described in absolute and percent frequencies and represented in Tables 1–3. To study the difference between categorical variables and their proportions, the chi-square test was used. Mann-Whitney and Student's t-tests were used to study the impact of the study group on continuous variables. Stepwise binary logistic regression analysis was used to assess the best predictors for early neonatal sepsis. The significance level for all the tests was set at $p<0.05$.

RESULTS

During the study period, 857 pregnant women were admitted, 229 of whom were excluded because they did not collect the swab for GBS culture and 7 cases were excluded due to missing information in the medical records. For the final statistical analysis, 201 cases with a positive culture for GBS and 420 cases with a negative culture were considered, indicating a 32.3% maternal colonization rate by GBS.

Table 1. Clinical characteristics of the study population.

	Positive culture (n=201)	Negative culture (n=420)	p-value
Age (years)	28.0 (23.0–34.5)	28.0 (24.0–34.0)	0.531†
Weight (kg)	78.0 (71.2–84.0)	77.5 (68.2–86.0)	0.532†
Height (m)	1.65 (1.62–1.68)	1.65 (1.60–1.69)	0.163†
BMI (kg/m²)	28.0 (26.3–31.2)	28.4 (25.9–31.3)	0.678†
Ethnicity			0.523§
White	46.2% (92/199)	49.3% (203/412)	
Black	11.1% (22/199)	8.3% (34/412)	
Mixed	41.2% (82/199)	41.7% (172/412)	
Asian	1.5% (3/199)	0.7% (3/412)	
Smoking	6.5% (13/200)	7.7% (32/417)	0.600§
Number of pregnancies	2.0 (1.0–3.0)	2.0 (1.0–3.0)	0.281†
Number of deliveries			0.030§
None	3.0% (6/201)	7.6% (32/420)	
≥1	97.0% (195/201)	92.4% (388/420)	
Gestational age at vaginal collection (weeks)	36.1 (35.8–36.4)	36.0 (35.6–37.0)	<0.001†
High-risk pregnancy	32.3% (65/201)	39.5% (166/420)	0.083§
Premature rupture of ovular membranes	13.9% (28/201)	20.0% (84/420)	0.066§
Preterm birth	4.5% (9/201)	7.1% (30/420)	0.200§
Gestational age at delivery (weeks)	39.4 (38.6–40.0)	39.3 (38.4–40.0)	0.332†
Antibiotic use prophylaxis	65.7% (132/201)	20.7% (87/420)	<0.001§
Types of delivery			0.006§
Vaginal	39.9% (79/198)	51.0% (212/416)	
Cesarean section	59.1% (117/198)	49.0% (204/416)	
Forceps	1.0% (2/198)	0.0% (0–416)	
Birth weight (g)	3270.0 (2933.0–3573.0)	3240.0 (2915.0–3520.0)	0.402†
Apgar score 1st minute	8.0 (8.0–9.0)	9.0 (8.0–9.0)	0.007†
Apgar score 5th minute	8.0 (8.0–9.0)	9.0 (9.0–10.0)	<0.001†

†Mann-Whitney test: median (interquartile range); §chi-square: percentage (N/n); p<0.05.

Pregnant women with a positive culture for GBS had a lower prevalence of nulliparity (3.0% vs. 7.6%, p==0.030), a higher prevalence of prophylactic antibiotic use (65.7% vs. 20.7%, p<0.001), and a higher prevalence of cesarean sections (59.1% vs. 49.0%, p==0.006) than pregnant women with negative GBS cultures (Table 1). Among pregnant women with negative GBS cultures, 71.3% (62/87) received prophylactic antibiotics due to cesarean section, 24.1% (21/87) due to PROM>18 h, and 4.6% (4/87) for urinary infection treatment.

There was no significant association observed between positive and negative cultures for GBS and the presence of early neonatal sepsis (p=0.399), APGAR score<7 at the 1st minute (p=0.081), neonatal ICU admission (p=0.802), neonatal death in the first 48 h (p=0.148), chorioamnionitis (p=0.489), and maternal ICU admission (p=0.645). The prevalence of early neonatal sepsis was 1.0% (2/201) among patients with a positive culture and 1.9% (8/420) among patients with a negative culture (p=0.399).

Pregnant women who received adequate prophylaxis had a significantly higher median number of doses than pregnant women who received inadequate prophylaxis (4.0 vs. 0.0, p<0.0001). Among the patients who received adequate prophylaxis, crystalline penicillin G was used in 51.9% (54/104), followed by cefazolin in 43.3% (45/104), ampicillin in 3.8% (4/104), and clindamycin in 1.0% (1/104). Antibiotic prophylaxis was not used in 70.1% (68/97) of patients with

Table 2. Clinical characteristics of pregnant women with a positive culture for B beta-hemolytic *Streptococcus* who received adequate or inadequate prophylaxis during labor.

	Adequate prophylaxis (n=104)	Inadequate prophylaxis (n=97)	p-value
High-risk pregnancy	35.6% (37/104)	28.9% (28/97)	0.309[§]
Number of antibiotic doses	4.0 (4.0–4.0)	0.0 (0.0–1.0)	<0.0001[†]
Antibiotic use			<0.001[§]
Crystalline penicillin G	51.9% (54/104)	20.6% (20/97)	
Ampicillin	3.8% (4/104)	3.1% (3/97)	
Clindamycin	1.0% (1/104)	2.1% (2/97)	
Cefazoline	43.3% (45/104)	4.1% (4/97)	
None	0.0% (0/104)	70.1% (68/97)	
Gestational age at delivery (weeks)	39.4 (38.9–40.1)	39.3 (38.3–40.0)	0.130[†]
Preterm birth	1.92% (2/104)	7.22% (7/97)	0.091[§]
Types of delivery			0.701[§]
Vaginal	42.7% (44/103)	36.8% (35/95)	
Cesarean section	56.3% (58/103)	62.1% (59/95)	
Forceps	1.0% (1/103)	1.1% (1/95)	
Birth weight (g)	3282 (489.7)	3197 (553.0)	0.253[£]
Apgar score at 1st minute	8.0 (8.0–9.0)	8.0 (8.0–9.0)	0.779[†]
Apgar score at 5th minute	9.0 (9.0–9.75)	9.0 (9.0–9.0)	0.728[†]

[£]Student's t-test: mean (standard deviation); [†]Mann-Whitney test: median (interquartile range); [§]chi-square test: percentage (N/n); p<0.05.

Table 3. Prediction of early neonatal sepsis, considering all cases included in the study, using positive culture for group B beta-hemolytic *Streptococcus*, inadequate prophylaxis, and prematurity as covariants.

	OR	CI 95%	p-value
GBS-positive	0.16	0.10–2.50	0.408
Inadequate prophylaxis	3.78	0.47–30.2	0.209
Prematurity	16.9	4.7–61.4	<0.001

OR: odds ratio; CI: confidence interval; GBS: group B beta-hemolytic *Streptococcus*; stepwise binary logistic regression; p<0.05.

inadequate prophylaxis. Among pregnant women with inadequate prophylaxis who received at least one dose of antibiotic, 20.6% (20/97) used crystalline penicillin G, 4.1% (4/97) used cefazolin, 3.1% (3/97) used ampicillin, and 2.1% (2/97) used clindamycin (Table 2).

There was no significant association between adequate and inadequate prophylaxis and adverse perinatal outcomes in pregnant women with a positive culture for GBS. There was no significant association between the groups regarding the presence of early neonatal sepsis (p=0.170), Apgar score at 1st minute<7 (p=0.671), neonatal ICU admission (p=0.654), neonatal death within first 48 h (p=0.333), chorioamnionitis, and maternal ICU admission (p=0.141). The prevalence of early neonatal sepsis was 1.9% (2/104) in patients with adequate prophylaxis and 0.0% (0/97) in patients with inadequate prophylaxis for GBS.

Considering all cases included in the study, a stepwise binary logistic regression model was created to assess whether positive culture for GBS, inadequate prophylaxis, and prematurity are predictors of early neonatal sepsis. The models including positive culture for GBS (p==0.408) and adequate prophylaxis for GBS (p==0.209) were not adequate to predict early neonatal sepsis, whereas prematurity proved to be an independent predictor (p==0.001). This model was significant in predicting the risk of early neonatal sepsis [χ^2 (1)==15.0, odds ratio (OR): 16.9, 95%CI 4.7–61.6, p<0.001, Nagelkerke R^2==0.157], with 98.4% prediction capacity (Table 3).

DISCUSSION

The universal bacteriological screening for GBS is controversial according to some scientific entities. The National Screening Committee of the United Kingdom does not recommend universal bacteriological screening for GBS[14]. Their view is that there is no clear evidence to show that testing for GBS routinely would do better than harm. The American College of Obstetricians and Gynecologists recommends a universal culture-based screening strategy for identifying candidates

for GBS intrapartum antibiotic prophylaxis, which has been demonstrated to be superior to risk-based screening protocols for the prevention of GBS early-onset disease[1]. In our study, the prevalence of a positive GBS culture was high. Even using antibiotic prophylaxis in pregnant women with a positive culture for GBS, we did not find a significant difference in early neonatal sepsis between groups. Prematurity was an independent predictor of early neonatal sepsis in our study population.

In a meta-analysis of maternal colonization rates by GBS in Africa, 83 articles were evaluated, of which 57 studies were conducted in 5 sub-regions in 21 countries (22,206 pregnant women). The overall rectovaginal colonization rate was estimated at 19.3%. The highest estimate was observed in South Africa (23.8%), followed by North Africa (22.7%), while the lowest was found in East Africa (15.4%)[15]. In Germany and Uruguay, maternal colonization rates by GBS were 16 and 67.3%, respectively[16,17]. In Brazil, a review of 21 articles found that the prevalence of maternal colonization by GBS ranged from 4.2 to 28.4% between 2008 and 2018, considering 3 geographical regions (South, Southeast, and Northeast) and 8 states[18].

In the present study, a high rate of maternal colonization by GBS was observed, probably due to the institution's protocols for universal screening using anal and vaginal swabs between 35 and 37 weeks and a high proportion of black and mixed people in the state of Minas Gerais (53.5%). The rate of maternal colonization by GBS is directly related to the screening rate. Out of six Latin American countries studied, Nicaragua presented the lowest rate (0.8%), while Uruguay had the highest rate (67.3%)[17]. In a study with 526 pregnant women with positive screening for GBS, black African ethnicity and sexually transmitted diseases were the only independent risk factors for maternal colonization by GBS[19]. Even within the same country, the prevalence of GBS colonization can vary widely. The main reason for this difference may be related to local economic levels and environmental factors. Another important factor is the neglect of the detection method for GBS.

In the present study, there was no significant association between positive and negative cultures for GBS and the presence of early neonatal sepsis. The prevalence of early neonatal sepsis was 1.0% (2/201) among patients with a positive culture for GBS and 1.9% (8/420) among patients with a negative culture. In a study conducted in South Korea, the prevalence of early neonatal sepsis was 1.5% (2/134) among patients with a positive culture for GBS and 0.3% (3/1,024) among patients with a negative culture[20].

Regarding adverse perinatal outcomes, no significant statistical differences were observed between the groups with positive and negative cultures for GBS. In a retrospective American study, Edwards et al.[21] estimated the prevalence of GBS colonization, compared the risk of adverse pregnancy outcomes by GBS colonization status, and estimated the incidence of invasive GBS disease. They found that overall 21.6% of the population was GBS colonized. In the adjusted analyses, there was an increased risk of gestational diabetes in colonized pregnancies and a decreased incidence of short cervix, chorioamnionitis, wound infection, and operative delivery. In a study in South Korea, pregnant women with a positive culture for GBS presented lower rates of preterm births without differences in PROM and intrauterine infection than those with a negative GBS culture[20]. In our study, the majority of pregnant women with GBS colonization routinely receive prophylactic intravenous antibiotics during labor. The resulting reduction in bacterial burden likely decreases the incidence of chorioamnionitis and wound infection rates and may decrease the risk of short cervix associated with subclinical infection.

In the present study, most pregnant women who underwent adequate prophylaxis used crystalline penicillin G (51.9%), followed by cefazolin (43.3%). However, antibiotic prophylaxis was not given to 70.1% of patients with inadequate prophylaxis. In a study in Ethiopia, most isolated GBS were sensitive to crystalline penicillin G and ampicillin, but erythromycin and clindamycin resistance were found in 50.0 and 40.9% of the isolated samples, respectively[22]. Of 3,494 GBS-positive cultures through a vaginal swab, penicillin resistance was observed in only 6 (0.2%). In a Chinese study, 636 (8.2%) of 7,726 pregnant women who were screened for GBS were positive, and 100% of this sample was sensitive to penicillin, which is recommended as the first choice for treatment and prevention of early neonatal sepsis[23]. These results are consistent with the findings of the present study, in which most patients received prophylaxis with crystalline penicillin G.

In our study, no reduction in the rate of neonatal sepsis was observed in patients who underwent prophylaxis for GBS. Most patients who underwent prophylaxis for GBS used crystalline penicillin and cefazolin, followed by ampicillin and clindamycin. We speculate that the lack of reduction in neonatal sepsis may be explained by different regimens of antibiotic prophylaxis used in our institution. The time-dependent bactericidal mechanism of action of β-lactam antibiotics supports the efficacy of ampicillin and penicillin administered at least 4 h before delivery[24]. No data specifically inform the clinical effectiveness of intrapartum antibiotic prophylaxis with cefazolin, but the pharmacokinetics and mechanism of bactericidal action for cefazolin are similar to those of penicillin and ampicillin that administration of cefazolin can be considered adequate prophylaxis against early-onset GBS. Although data on the

pharmacokinetics of clindamycin and vancomycin have been published, evidence on their clinical efficacy is more limited. Therefore, when non-β-lactam antibiotics of any duration are administered for intrapartum antibiotic prophylaxis of GBS, such treatment should be considered not fully adequate for neonatal risk assessment purposes[24].

Regarding inadequate treatment, in an Italian study, out of 136 pregnant women with an indication for antibiotic prophylaxis use, only 68 (50%) received adequate treatment[10]. This inadequate prophylaxis rate is very similar to that observed in the present study, which was 48.2%. In a French study, 5,997 pregnant women were evaluated between 2006 and 2008, and the GBS colonization rate ranged from 13 to 18%. In that study, it was observed that the percentage of pregnant women who received correct antibiotic prophylaxis remained stable during the period[25]. In the present study, despite the high rate of inadequate prophylaxis, there were no cases of early neonatal sepsis in this group. Inadequate prophylaxis may contribute to an increased early neonatal sepsis rate and may be explained by the higher incidence of women in advanced labor, making it difficult to fully implement the antibiotic therapy protocol.

Failure to diagnose neonatal sepsis quickly, primarily due to its vague signs and symptoms, makes the disease more deadly and destructive. A blood culture report, as the only main solution, takes practically 2 days to generate a result. Therefore, there is a need to look into novel approaches that can help in the rapid prediction of neonatal sepsis. In the present study, a binary logistic regression model was created, which showed that prematurity is an independent predictor of early neonatal sepsis. Spaans et al.[11] studied 8,215 births between 1983 and 1988 and observed 104 cases of pneumonia and 50 cases of sepsis. Cultures for GBS were positive in 46% of neonatal sepsis cases. After testing all risk factors identified by univariate analysis in a logistic regression model, tachycardia remained an independent predictor of neonatal pneumonia or sepsis.

CONCLUSION

The prevalence of positive culture for GBS was high. However, the prevalence of early neonatal sepsis was low in pregnant women with both positive and negative GBS cultures and in pregnant women with a positive culture who underwent both adequate and inadequate antibiotic prophylaxis. Prematurity proved to be an independent predictor of early neonatal sepsis, considering the entire study population.

AUTHORS' CONTRIBUTIONS

ABP: Conceptualization, Formal Analysis, Project administration, Supervision, Visualization. **JAVR:** Conceptualization, Visualization. **CGCS:** Data curation, Visualization, Writing – original draft. **MJAL:** Data curation, Visualization, Writing – original draft. **APMF:** Investigation, Visualization. **JTM:** Methodology, Visualization. **EAJ:** Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Changes in ghrelin, GLP-1, and PYY levels after diet and exercise in obese individuals

Gülşah Alyar[1*], Fatma Zuhal Umudum[2], Nergis Akbaş[3]

SUMMARY

OBJECTIVE: Diet and exercise, which are the building blocks of obesity management, provide weight loss by creating a negative energy balance. However, the effect of energy deficit induced by long-term diet and exercise on appetite hormones remains unclear. The study was designed to determine the effect of a 12-week diet and exercise program applied to obese individuals on the levels of appetite hormones, namely, ghrelin, GLP-1, and PYY.

METHODS: A total of 62 obese individuals (BMI≥30) and 48 healthy controls (BMI 18.50–29.99) participated in the study. Appropriate diet (1000–1500 kcal/day) and exercise (at least 5000 steps/day) programs were applied to obese individuals according to age, gender, and BMI. The ghrelin, GLP-1, and PYY values of the participants were analyzed by the ELISA method and commercial kit by taking venous blood samples before and after 12 weeks of treatment.

RESULTS: While ghrelin levels of individuals decreased significantly after diet and exercise, PYY levels increased significantly. However, despite the treatment applied, the GLP-1 and PYY levels of the case group did not reach the levels of the control group.

CONCLUSION: Long-term diet and exercise intervention had a positive effect on appetite regulation hormones. It reduced ghrelin levels after treatment. Associated weight loss was facilitated. In the case group, increased satiety hormones after combined treatment supported the maintenance of body weight by increasing satiety.

KEYWORDS: Calorie restriction. Exercise. Ghrelin. GLP-1. Obesity. Peptide YY.

INTRODUCTION

The prevalence of overweight and obesity is increasing dramatically around the world. Obesity, which has a complex development with a multifactorial etiology, is mainly caused by positive energy homeostasis[1]. Dietary and exercise interventions, which form the basis of obesity management, reduce body weight by creating a negative energy balance[2]. However, compensatory metabolic and behavioral adaptations in appetite and energy intake in energy deficit may reduce treatment efficacy and lead to weight gain[3]. Acute calorie restriction has been shown to increase hunger and support food intake with an increase in orexigenic signals and a decrease in anorexigenic signals in both obese and normal-weight individuals[4,5]. In contrast, exercise alone can attenuate as well as limit compensatory changes in appetite and energy intake[3]. In fact, it has been determined that the increased energy deficit due to exercise intensity decreases food intake by decreasing hunger hormones and increasing satiety hormones[6]. However, the effect of long-term diet and exercise intervention on appetite hormones in obese individuals remains unclear. Our study was designed to determine the effect of a 12-week diet and exercise applied to obese individuals on ghrelin, GLP-1, and PYY levels. Ghrelin, GLP-1, and PYY are appetite modulators that regulate food intake by mediating neuroendocrine control of energy homeostasis[7]. Ghrelin is a 28-amino acid orexigenic hormone produced extensively by the stomach. As the only stimulator of peripheral food intake, ghrelin has many biological effects, such as stimulating the release of growth hormone and regulating glucose and lipid metabolism[8]. GLP-1 and PYY are anorexigenic hormones secreted from intestinal cells in response to food intake. Satisfaction hormones not only reduce intestinal motility by inducing the "ileal brake" mechanism but also provide appetite and body weight control by acting on the brain stem[9].

METHODS

The study, which was approved by the Atatürk University Clinical Research Ethics Committee's decision numbered B.30.2.ATA.0.01.00/31, was carried out in the laboratory of Atatürk University Faculty of Medicine, Department of Medical Biochemistry.

[1]Atatürk University, Vocational School of Health Services – Erzurum, Turkey.
[2]Atatürk University, Faculty of Medicine, Department of Medical Biochemistry – Erzurum, Turkey.
[3]Yalova University, Faculty of Medicine, Department of Basic Medical Sciences – Erzurum, Turkey.
*Corresponding author: gulsah.kiymik@atauni.edu.tr
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Materials

The minimum number of samples required for the study was calculated by G Power analysis (version 3.1.9.4) at the level of Type I error (α) 0.05 and Type II error (1–β) 0.85. Accordingly, the t-test in independent groups was determined as at least 24 individuals (Cohen's f: 0.8) for each group, and the t-test in dependent groups was determined as 48 individuals (Cohen's f: 0.8) in each group[10]. The study consisted of individuals who applied to the Erzincan Obesity Center between September 2022 and December 2022. The inclusion criteria for the case group were adult subjects with BMI≥30 kg/m^2, diabetes mellitus, psychological disorder, and undiagnosed disease that could affect appetite. Exclusion criteria were smoking, psychiatric disorder, and use of any medication with an effect on the appetite mechanism. Inclusion and exclusion criteria for healthy controls consistent with the case group in terms of age and sex were the same, except for having a normal weight (BMI 18.50–29.99). The volunteers were informed about the study, read the "Informed Voluntary Consent Form," and were included in the study after obtaining approval.

Anthropometric measurements

The BMI of the groups was calculated by dividing the body weight (kg) by the square of the height in meters. Using the BMI classification of the World Health Organization, those with a BMI between 18.50 and 24.99 kg/m^2 were normal (n=48), and those with a BMI of ≥30 kg/m^2 were included in the obese (n=62) group.

Method

In our study, a diet (1000–1500 kcal/day) and physical activity (at least 5000 steps/day) program suitable for age, gender, and BMI parameters was created for the case group. The values of appetite hormones, namely, ghrelin, GLP-1, and PYY in serum samples of the case group were analyzed by the ELISA method and commercial kit by taking venous blood samples before and 12 weeks after starting the diet and physical activity program.

RESULTS

A total of 100 individuals with 62 obese and 48 normal body weight were included in the study. PYY values increased significantly after the 12-week treatment program applied to the obese. PYY levels before and after the treatment program are shown in Figure 1. In addition, it was determined that the levels of the hunger hormone ghrelin decreased after the treatment, while the levels of the satiety hormone GLP-1 increased. The ghrelin levels before and after the treatment are shown in Figure 2, and the GLP-1 levels are shown in Figure 3. In addition, post-treatment fasting and satiety hormone levels were found to be lower compared with the control group.

The saturation hormone PYY levels after the treatment program applied to the case group are shown in Figure 1.

The change in the satiety hormone GLP-1 concentration after the treatment program applied to the case group is shown in Figure 2.

The change in ghrelin hormone after the treatment program applied to the case group is shown in Figure 3.

DISCUSSION

Sustainable weight loss may not always be achieved in the energy deficit created by lifestyle changes (calorie restriction and exercise)[11]. This study evaluates the effect of long-term diet and exercise-induced

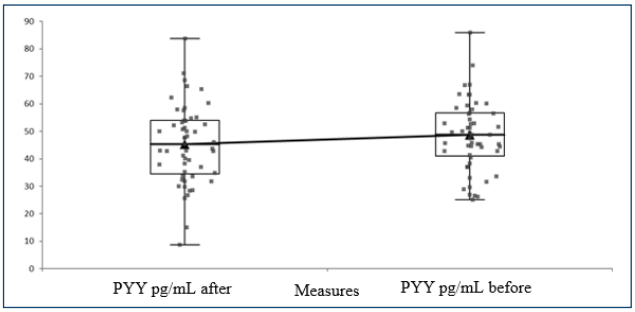

Figure 1. PYY levels before and after 12 weeks of treatment.

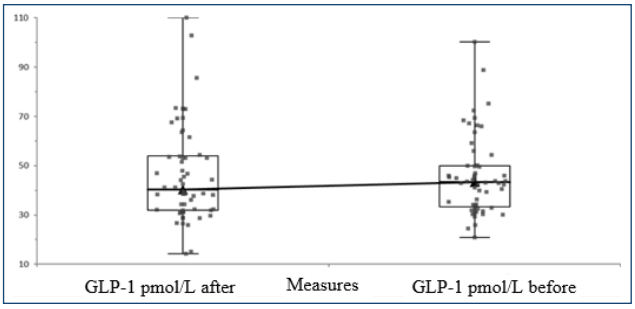

Figure 2. GLP-1 levels before and after 12 weeks of treatment.

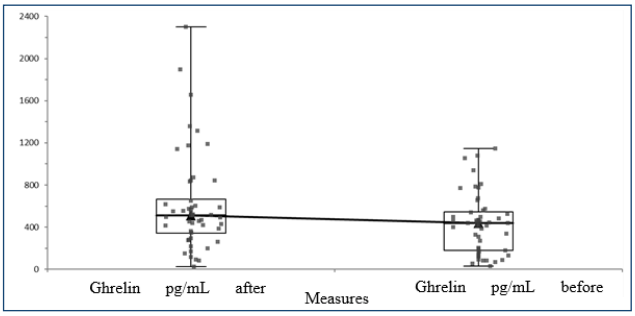

Figure 3. Ghrelin levels before and after 12 weeks of treatment.

energy deficit on appetite responses in obese individuals. The appetite-stimulating hormone ghrelin negatively correlates with body weight, while it positively correlates with a low-calorie diet[1]. Similarly, in our study, ghrelin levels of the obese were significantly lower compared with the control group. One of the important findings of our study was that ghrelin levels decreased significantly after the diet and exercise program was applied to obese adults. In parallel with our results, it was determined that ghrelin levels decreased after weight loss with long-term diet and exercise intervention[8]. However, dietary and exercise interventions can initially increase the release of ghrelin triggering an appetite-enhancing response to meet the body's energy needs[12]. The first study on the subject investigated the effects of diet and exercise on food intake and appetite in acute energy deficit induced by diet and exercise in healthy individuals. Accordingly, while the feeling of hunger and food consumption increased in food restriction, perceived hunger and food intake did not change in the energy deficit created by moderate exercise[13]. A study investigating longer (3-day) compensatory responses to diet and exercise in a similar population found that ghrelin levels were similarly elevated. In the same study, PYY3-36 levels were lower in the diet group compared with the exercise group[14]. A study comparing food intake in the equivalent energy deficit induced by diet or exercise in obese adolescents showed an *ad libitum* increase in both acute exercise and dietary restriction. In addition, as a result of the study, a negative correlation was observed between the degree of deficiency induced on the exercise day and energy intake, while a positive correlation was observed with the amount of calories consumed in the *ad libitum* meal on the diet day[15]. In a recent study, the feeling of hunger and food intake in the acute energy deficit created by exercise in adolescents with obesity were higher in the diet group compared with the control and exercise groups[12]. These results suggest that appetite perceptions are sensitive to diet-induced acute energy deficit. However, short-term exercise was not effective in stimulating food intake and appetite, but attenuated compensatory responses. On the contrary, unlike previous studies, our study investigated the effects of a long-term (12-week) diet and exercise intervention applied to obese individuals on both orexigenic and anorexigenic signals. Anotherimportant finding of our study was that PYY levels increased significantly after combined treatment. However, although GLP-1 and PYY levels increased after treatment, they did not reach the levels of those in the control group. The increase in satiety hormone levels was associated with gastrointestinal motility and free fatty acid concentration changes caused by calorie restriction as well as blood redistribution with exercise sympathetic nervous system activity, cytokine release, and lactate production. In a study examining the changes in appetite hormones of intermittent exercise and short-term calorie restriction in obese women, it was determined that fasting PYY levels did not change postprandial PYY increase and desacyl ghrelin decrease. It has been suggested that the energy deficit created by the study treatment program partially affects PYY, strongly suppressing acylated ghrelin[16]. The study that evaluated the effect of long-term fasting and subsequent aerobic exercise on hunger and satiety hormone release found that the level of ghrelin decreased and the secretion of satiety hormone increased[17]. In contrast, Adam et al., studied the effect of a 6-week low-calorie diet on GLP-1 levels in overweight/obese individuals. Contrary to our findings and studies consistent with these results, basal and postprandial GLP-1 levels decreased after weight loss in the study[18]. Accumulating evidence suggests that changes in appetite hormone concentrations depend on the intensity, type, and duration of exercise as well as the study population[2]. Increasing exercise intensity regulates appetite and energy intake by suppressing hunger hormones and stimulating satiety hormones[19]. The study that evaluated the effect of very low volume exercise on appetite hormones in overweight individuals reported that the ghrelin levels of the individuals who exercised were lower than the control, but their GLP-1 levels were higher[20]. The study examining the acute effects of moderate-intensity exercise on appetite hormones in overweight/obese individuals reported that moderate-intensity exercise temporarily inhibited appetite and stimulated PYY and GLP-1 in this population. However, the study found that it did not induce compensatory changes in appetite or energy intake in underweight and overweight/obese subjects[21]. The study that examined the relationship between intense physical exercise and appetite regulation in normal and overweight individuals concluded that PYY was stimulated and ghrelin was suppressed after exercise, and it was suggested that exercise would reduce energy intake[22]. In a study investigating the effect of exercise intensity on appetite and food intake in men with normal body weight, nutrient intake after high-intensity exercise was found to be lower than after moderate-intensity exercise. The ghrelin levels of sedentary controls were found to be higher than those who exercised. As a result, it has been reported that exercise reduces appetite and restricts food intake in this population[19]. In the study investigating the gender-based differences in appetite and energy intake in exercise-induced energy deficit, no statistical difference was observed in ghrelin, PYY, and GLP-1 levels between the sexes after exercise in overweight/obese men and women[23]. In line with all these results, the application of calorie restriction and exercise together helps control weight by having a positive effect on appetite and energy regulation.

CONCLUSION

The energy deficit induced by long-term diet and exercise positively affected appetite and energy regulation by

inhibiting the hunger hormone and stimulating the satiety hormones. Due to the decreased ghrelin levels after the treatment, appetite was suppressed and weight loss was facilitated. After the combined treatment, increased satiety hormones supported the maintenance of body weight by increasing satiety.

AUTHORS' CONTRIBUTIONS

GA: Conceptualization, Formal Analysis, Investigation, Project administration, Writing – original draft, Writing –review & editing. **FZU:** Conceptualization, Formal Analysis, Investigation, Project administration, Writing – original draft, Writing –review & editing. **NA:** Data curation.

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ORIGINAL ARTICLE

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Effects of low-level laser therapy on acupuncture points on knee pain and function in knee osteoarthritis

Ayşegül Yetişir[1]*, Gülşah Yaşa Öztürk[2]

SUMMARY

OBJECTIVE: Knee osteoarthritis is a common and disabling disease. We aimed to examine the effect of low-level laser therapy in addition to routine physical therapy modalities (transcutaneous electrical stimulation, superficial heating modality of infrared, ultrasound, and exercise) on the functional status and pain in knee osteoarthritis.

METHODS: Patients with knee osteoarthritis (n=71) who underwent physical therapy (transcutaneous electrical stimulation, infrared, ultrasound, exercise therapy, and low-level laser therapy) were retrospectively screened. Patients who received low-level laser therapy on acupuncture points, transcutaneous electrical stimulation, infrared, ultrasound, and exercise were included in the low-level laser therapy (+) (n=35), and patients who received only transcutaneous electrical stimulation, ultrasound, infrared, and exercise were included in the low-level laser therapy (–) group (n=36). The Visual Analog Scale for activity pain, Lysholm Knee Scoring Scale, and walking and stair climbing tests were used before and after treatment obtained from patient files.

RESULTS: The post-treatment Visual Analog Scale activity score and walking and stair climbing test results were statistically significantly lower in the low-level laser therapy (+) group than in the low-level laser therapy (–) group. There was no significant difference in post-treatment Lysholm Knee Scoring Scale scores between the two groups. In both groups, the Visual Analog Scale activity, Lysholm Knee Scoring Scale, and walking and stair climbing test scores statistically significantly decreased after treatment.

CONCLUSION: Knee osteoarthritis increases with aging and creates significant functional limitations. low-level laser therapy with routine physiotherapy contributed to the improvement in the pain and functional status of the patients with knee osteoarthritis. Low-level laser therapy can be recommended in osteoarthritis treatment guidelines with the support of further studies, which is an easy-to-apply, effective, and reliable method.

KEYWORDS: Osteoarthritis. Knee. Physical therapy modalities. Laser therapy.

INTRODUCTION

Traditionally, osteoarthritis has been considered to be the progressive wear and tear of articular cartilage. Recent evidence has shown that osteoarthritis is an inflammatory disease involving not only the mechanical degeneration of articular cartilage but also the structural and functional alteration of the entire joint, including the synovium, meniscus (in the knee), periarticular ligament, and subchondral bone[1]. In the adult population, the incidence of structural and symptomatic knee osteoarthritis (KOA) is 6%, which increases with age, reaching rates of up to 40% in individuals aged 70–74 years[2]. KOA is one of the leading causes of pain and disability across the world and reduces quality of life[3]. Treatment options for KOA include patient education, exercise, lifestyle modifications such as weight control, orthoses, physical therapy applications, pharmacotherapy, intra-articular methods, and surgery[4]. Available traditional treatments with limited efficacy are pharmacotherapy and physiotherapy[5].

The goal of osteoarthritis treatment is to slow the progression of the disease by reducing symptoms. This can also reduce the negative effect of osteoarthritis on the patient's mobility and quality of life[6]. In the 2019 American College of Rheumatology (ACR) guidelines, transcutaneous electrical stimulation (TENS) is highly recommended in KOA[7]. According to the 2014 European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis, and Musculoskeletal Diseases, superficial heating is recommended in treatment at any time when osteoarthritis is symptomatic[6]. Therapeutic ultrasound (US) is a safe and effective treatment for relieving pain and functional improvement in KOA[8]. Acupuncture treatment for KOA has gained popularity in recent years. Acupuncture is an effective drug-free treatment with few side effects and low cost[9]. In the literature, there are also laser applications on acupuncture points[10]. Low-level laser therapy (LLLT) is known to induce the anti-inflammatory process[11]. It has a stimulating effect on the inflammatory process

[1]Çukurova University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Division of Rheumatology – Adana, Turkey.
[2]Adana City Training and Research Hospital, Department of Physical Medicine and Rehabilitation – Adana, Turkey.
*Corresponding author: aybegul8686@hotmail.com
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and tissue metabolism after injury. It increases cellular oxygenation and mediates the release of neurotransmitters associated with pain modulation and the release of anti-inflammatory endogenous mediators[12]. However, LLLT is not recommended in major osteoarthritis treatment guidelines. There are studies showing positive and negative results concerning the efficacy of LLLT in the treatment of KOA[11,12]. In this study, we aimed to investigate the effects of biostimulant, analgesic, and non-invasive LLLT applied to acupuncture points in addition to routine physical therapy modalities (superficial heating modality of infrared (IR), TENS, US, and exercise) on the pain, functionality, and quality of life of patients with KOA.

METHODS

Patients with KOA who underwent TENS, superficial heating modality of IR with heat lamps, US, and exercise therapy with and without the addition of LLLT on acupuncture points for 15 treatment sessions were retrospectively observed. Patients, aged 40–75 years, who were administered the Visual Analog Scale (VAS) for activity pain, Lysholm Knee Scoring Scale (LKSS), and walking and stair climbing tests before and after treatment were included in file screening.

Exclusion criteria:

1. Not completing 15 sessions of physical therapy
2. Pregnancy and breastfeeding
3. Having received any physical therapy within the last 6 months or having used analgesics or antimuscarinic agents in the past
4. Peripheral vascular disease
5. Type 2 diabetes mellitus
6. History of fracture or surgery in the knee area
7. Inflammatory rheumatological disease
8. Cancer history
9. Acute inflammation

The files of the patients were screened, and their age, gender, weight, height, comorbidities, pretreatment, and post-treatment VAS activity scores, walking and stair climbing test results, and LKSS scores were recorded by a physiatrist. The physiatrist was blind to the groups in which the patients were included.

The patients (n=71) were divided into two groups according to whether they received LLLT on acupuncture points in addition to TENS, IR, US, and exercise therapy. Patients who completed the physical therapy program for 15 sessions were randomly selected from the groups. There were 36 patients in the LLLT (–) group and 35 patients in the LLLT (+) group. The LLLT (–) group did not receive a placebo LLLT. In both groups, the patients received a total of 15 treatment sessions over 3 weeks. In both groups, 20-min IR, 6-min US 1.5 W/cm^2, and 20-min TENS were applied. In addition, all the patients were given a home exercise program, including quadriceps strengthening, isometric, and isotonic exercises, and instructed to perform 10 repetitions of each exercise every day. In the LLLT (+) group, in one session, a patient was given a total dose of 25 mW (gallium arsenide laser, 904 nm wavelength, 4J/point). Treatment was administered in skin contact to six acupuncture points on the knee (ST34, ST35, GB34, SP10, EX-LE4, and SP9) for 180 s.

Statistical analysis

Continuous variables were presented as mean±standard deviation and median (min–max) values, and categorical data were presented as numbers and percentages. In the inter-group analysis of continuous variables, the normality of data distribution was examined with the Kolmogorov-Smirnov goodness-of-fit test. Student's t-test was used to compare the data that fit the normal distribution, and the Mann-Whitney U test was used for non-normally distributed data. The comparisons of categorical data were made with the chi-square test. The Wilcoxon signed-rank test was used for the intra-group analysis. Statistical analyses for 71 patients' data were performed using IBM SPSS version 26.0 (IBM Corporation, Armonk, NY, USA). The statistical significance level was accepted as $p<0.05$.

Ethics

The study was approved by the Clinical Research Ethics Committee of our hospital with decision number 1828, dated 10.03.2022.

RESULTS

A total of 71 patients were examined in this study, of which 36 were in the LLLT (–) group and 35 were in the LLLT (+) group.

There was no statistically significant difference between the LLLT (+) and (–) groups in terms of age, mean body mass index, gender, and rate of comorbidities ($p>0.05$) (Table 1).

The pretreatment VAS activity and LKSS scores and walking and stair climbing test measurement values did not significantly differ between the treatment groups ($p>0.05$). However, the LLLT (+) group had statistically significantly lower post-treatment VAS scores and walking and stair climbing test measurement values compared with the LLLT (–) group ($p<0.001$ for all). The mean post-treatment LKSS score was also lower in the LLLT (+) group, but this was not statistically significant compared with the LLLT (–) group ($p=0.201$) (Table 2).

Table 1. Comparison of the demographic and clinical characteristics of the groups.

	LLLT (–) (n=36)	LLLT (+) (n=35)	p
Age (years) (mean±SD)	63.0±9.2	66.6±7.1	0.063*
BMI (kg/m²) (mean±SD)	27.8±2.7	26.6±2.6	0.087*
Gender (n, %)			
Female	22 (61.1)	20 (57.1)	0.734*
Male	14 (38.9)	15 (42.9)	
Comorbidity (n, %)			
Absent	18 (50.0)	17 (48.6)	0.904*
Present	18 (50.0)	18 (51.4)	

*Student's t-test. LLLT: low-level laser therapy; BMI: body mass index.

Table 2. Intra-group and inter-group comparison of the pretreatment and post-treatment evaluations of the groups.

	LLLT (–) (n=36) [median (min–max)]	LLLT (+) (n=35) [median (min–max)]	p
VAS activity (pretreat)	8 (7–10)	8 (7–10)	0.631*
VAS activity (posttreat)	4.5 (2–8)	3 (2–5)	**<0.001***
	p<0.001**	**p<0.001****	
LKSS (pretreat)	76.5 (45–90)	78 (55–90)	0.719*
LKSS (posttreat)	52.5 (20–85)	45 (30–65)	0.201*
	p<0.001**	**p<0.001****	
Walking test (pretreat)	80 (40–110)	75 (40–90)	0.187*
Walking test (posttreat)	57.5 (30–100)	45 (25–65)	<0.001*
	p<0.001**	**p<0.001****	
Stair climbing test (pretreat)	65 (35–90)	65 (40–85)	0.333*
Stair climbing test (posttreat)	50 (15–80)	40 (25–65)	<0.001*
	p<0.001**	**p<0.001****	

*Mann-Whitney U test. **Wilcoxon signed-rank test. LLLT: low-level laser therapy; VAS: Visual Analog Scale; pretreat: pretreatment; posttreat: post-treatment; LKSS: Lysholm Knee Scoring Scale.

In intra-group evaluations, it was determined that the VAS activity and LKSS scores and walking and stair climb values statistically significantly decreased in the post-treatment period compared with the pretreatment period in both groups (p<0.001) (Table 2).

DISCUSSION

Knee osteoarthritis is a disease that increases with age and causes pain, functional limitation, and decreased quality of life. Among the many methods that can be preferred in the treatment of KOA, LLLT is a non-invasive option, but its efficacy remains controversial. In a systematic review and meta-analysis evaluating the effect of LLLT on pain and disability in KOA, LLLT was reported to significantly reduce pain and disability compared with the placebo[11]. In a randomized controlled study including 215 patients with KOA, the combination of stretching exercises with laser therapy was found to improve pain at rest, activities of daily living, stiffness, muscle contracture, and range of motion[13]. Liao et al., investigated the efficacy of dual-frequency LLLT (combination of red light at 780 nm and near-IR light at 830 nm) in 30 patients with KOA. The authors applied LLLT and placebo laser therapy to three acupuncture points (SP9, SP10, and EX-LE2) on the knee joints and reported that the application of dual-frequency LLLT to these acupuncture points reduced pain and disability in KOA[5]. In a study in which active laser acupuncture and placebo were compared in KOA, 10 sessions of treatment were applied using a gallium aluminum arsenide laser device on the ST35, ST36, SP9, GB34, and EX-LE4 acupuncture points on the affected knee. In the laser acupuncture group, the VAS scores showed significant improvement compared with the placebo group[14]. A total of 40 patients with bilateral grade 2 KOA were evaluated in two groups. In the first group, 5.4 joule laser was applied to the acupuncture points (ST35, ST36, SP9, SP10, and GB34) in each session. Acupuncture points were applied for 1 min in each session, and a total of 12 sessions were treated. The patients of the second group are the control group and have received sham laser. In group 1, VAS decreased, serum beta-endorphin levels increased, and serum substance p levels decreased compared with the control group after treatment[15]. Groups were designated as no acupuncture (control group, n=71) and needle acupuncture (n=70), laser (n=71), and sham laser (n=70) acupuncture. Neither needle nor laser acupuncture has significantly improved pain and functionality compared with shame. They commented that the findings do not support acupuncture in patients with moderate to severe chronic knee pain[16]. In a study investigating the safety and efficacy of LLLT in KOA, Rayegani et al., found LLLT to be superior to the placebo in terms of rest, activity, and total pain scores, and the Western Ontario and McMaster Universities Osteoarthritis Arthritis Index (WOMAC) function, stiffness, and total scores. However, in that meta-analysis, the authors stated that they did not have data on how LLLT efficacy was affected by wavelength, energy density, treatment duration, number of sessions, treatment, osteoarthritis severity, and application site[17]. In another study, a total of eight sessions of LLLT (830 nm) were applied to 4 points (20.1 J/cm² per point) in 35 patients with KOA, and significant improvement was found in VAS scores at the end

of the treatment[18]. LLLT (904 nm, 10 mW/cm^2 power density) and placebo laser were compared in patients with grade 2 and 3 KOA. Pain on movement (pVAS), 50-foot walking time (50 foot w), knee circumference (KC) improved significantly in the LLLT group. In the placebo group, significant improvement was observed in pVAS, 50 foot w, and WOMAC. When the two groups were compared, the improvement in KC was more significant in the LLLT group at 2 weeks. As a result, LLLT was said to be effective only in reducing periarticular swelling[19]. In contrast, in a systematic review and meta-analysis evaluating nine randomized controlled trials, LLLT was not found to be effective for KOA. In this study, seven randomized controlled trials using sham laser versus LLLT were reviewed. There was no significant difference between LLLT and sham in VAS scores within 2 weeks of treatment. All five studies evaluated delayed (12 weeks) outcomes, and no difference was observed in VAS scores. WOMAC pain, stiffness, and function values were not different between the groups immediately after treatment in five studies and 12 weeks after treatment in three studies. It was stated that LLLT had no positive effect on pain and functionality either in the early or late period[12]. Stausholm et al., stated that there was a methodological error in the meta-analysis of Huang et al. Statistical analysis of repeat data showed that there was a significant improvement in VAS values in favor of the LLLT group compared with placebo[20]. Atalay et al., who applied a total of 12 sessions of hot pack application, US, TENS, and home exercise program to the physiotherapy group, reported significantly lower VAS and WOMAC function scores at the end of treatment and at 12 weeks after the end of treatment. The WOMAC total and pain scores did not significantly change at the end of treatment, but they were significantly lower at 12 weeks after the end of treatment[21]. In our study, statistically significant results were observed in the post-treatment VAS activity, LKSS, and walking and stair climbing test scores of both groups compared with the pretreatment values. When the post-treatment values of the groups were compared, no statistically significant difference was found in the LKSS score, but a statistically significant decrease was detected in the VAS activity score and walking and stair climbing test results in favor of the LLLT (+) group. TENS, IR, US, and exercise, which are used in the routine treatment of KOA, are known to be effective, but they do not prevent the recurrence of patient symptoms. In this study investigating the effect of LLLT added to routine treatment, we observed better results in the LLLT (+) group compared with the group that only received routine physiotherapy. LLLT can be added to physiotherapy in appropriate patients as an easy-to-apply method with a very low side-effect profile. Our study is retrospective, and there are no long-term results. LLLT and routine physical treatments of the patients were not applied by the same physiotherapist. These are our study's limitations.

ETHICS COMMITTEE

The study was approved by the Clinical Research Ethics Committee of our hospital with decision number 1828, dated 10.03.2022.

AUTHORS' CONTRIBUTIONS

AY: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **GYÖ**: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Morbimortality and determinants of reperfusion in ischemic stroke

Adriana Ribeiro Oliveira[1], Pedro Antônio Pereira de Jesus[2], Fábio Vieira de Bulhões[1], Eduardo Martins Netto[1], Jamary Oliveira-Filho[1,2], Leonardo Roever[3], Alex Cleber Improta-Caria[1*], Roque Aras[1]

SUMMARY

BACKGROUND: Cerebrovascular accident (or stroke) and ischemic heart disease are the the major causes of death in the world. It is estimated that about 85% of strokes are ischemic in origin. Reperfusion therapy in the acute phase of ischemic stroke with a recombinant human tissue plasminogen activator is effective, but some factors influence the success of this treatment.

OBJECTIVE: The aim of this study was to evaluate clinical aspects and possible determinants for reperfusion after venous thrombolysis.

METHODS: This is a retrospective, cross-sectional, observational study based on a review of hospital records of inpatients diagnosed with ischemic stroke treated with intravenous thrombolysis, the main outcome being reperfusion or not.

RESULTS: Data from this study revealed a predominance of females in the group of reperfused patients and males in the non-reperfused group, both maintaining moderate severity on the National Institutes of Health Stroke Scale and admission without statistical significance (p>0.18). In addition, the mean admission severity score was 13.2 for the group of reperfused patients and 14.2 for those not reperfused, and the mean ejection fraction of both groups was within normal functionality, with a mean of 0.50 for reperfused patients and 0.62 for non-reperfused patients.

CONCLUSION: We found an association between successful venous chemical thrombolysis reperfusion and lower mortality in patients with acute stroke.

KEYWORDS: Acute ischemic stroke. Acute ischemic stroke. Thrombolysis. Reperfusion.

INTRODUCTION

Cerebrovascular accidents (CVA or stroke) and ischemic heart diseases are the major causes of death worldwide, with over 2 million deaths since the year 2000 and almost 9 million in 2019. Apart from causing premature deaths, CVA is also one of the main diseases compromising the physical capacity of individuals in their daily activities. Data from the World Health Organization (WHO) demonstrate that the CVA is the second cause of death in adults around the world and the main cause in Brazil, being responsible for 10% of total amount of deaths, 32.6% of deaths of vascular origin, and 40% of early retirements[1,2].

The American Stroke Association describes the CVA as an acute focal injury of the central nervous system by a vascular cause or even with clinical evidence of symptoms persisting for over 24 h and exclusion of other etiologies. Accordingly, the CVA is characterized by the sudden loss of blood circulation (thrombotic or embolic arterial obstruction) in a determined area of the encephalon with corresponding symptoms of loss of focal or global brain functions, possibly leading to severe cases of coma or not[3]. It is estimated that around 85% of cerebrovascular accidents have ischemic origin (I-CVA) and 15% are hemorrhagic. I-CVA occurs due to thrombosis and is responsible for 30% of the cases of CVA, with high risk considered for individuals with arterial fibrillation, thrombi in the left ventricle, cardiac tumors, valve diseases, expanded myocardiopathy, and heart failure[4].

Thrombolytic treatment with recombinant tissue plasminogen activator (rtPA) during the acute phase of the I-CVA is evidence level 1A[5]. The venous use of the rtPA is time-dependent and decreases morbidity by 30% when applied within a period £4.5 h[6].

Treatment during the acute phase aims to re-establish the flow in the occluded vessel in the lowest possible timeframe and reduce oxygen consumption in the areas at risk. The therapeutic evolution of the disease follows similar steps for the treatment of acute myocardial infarction, initially adopting measures to protect the cells submitted to the Ischemia through a reduction in metabolic consumption, while taking measures to re-establish the flow[6,7]. Considering the high rates of individuals still afflicted by acute I-CVA and the effects resulting from the non-clearance of the interruption of the secondary

[1]Universidade Federal da Bahia, Post-Graduate Program in Medicine and Health – Salvador (BA), Brazil.
[2]Roberto Santos General Hospital, Neurology Service – Salvador (BA), Brazil.
[3]Lebanese American University, Gilbert and Rose-Marie Chagoury School of Medicine – Beirut, Lebanon.
*Corresponding author: aleximprotacaria@gmail.com
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blood flow to possible cardioembolic cardiac alterations, the object of this study was to assess the clinical aspects and possible determinants for reperfusion after venous thrombolysis in the I-CVA[7].

METHODS

This is a retrospective observational cross-sectional study, based on a review of hospital records of admitted patients with a diagnosis of ischemic CVA treated with intravenous thrombolysis, with the main outcome being the occurrence of reperfusion. Clinical and demographic variables, comorbidities, and findings of the transthoracic echocardiogram performed in post-thrombolysis were analyzed. The study was carried out in a large-sized public high-complexity hospital, certified by the Ministry of Health and Education as a teaching hospital and hospital of reference in neurological and neurosurgical emergencies, being the only public hospital available with open doors to venous thrombolysis in the state.

The selected patients were those hospitalized consecutively with the diagnosis of acute I-CVA and who were submitted to venous thrombolytic treatment at the CVA unit of reference and had met the inclusion criteria. The convenience sample was of the non-probabilistic type. Patients above the age of 18 years were included in I-CVA, diagnosed, and submitted to venous thrombolysis at the unit specialized in acute cerebrovascular diseases in the period between January 2015 and December 2018. Patients under the age of 18 years and over 80 years, as well as patients with coagulopathy, prior hemorrhagic CVA, I-CVA with prior hemorrhage, I-CVA with pre-thrombolysis hemorrhagic transformation, previous background of some form of intracranial hemorrhage or cerebral vascular malformation, or contraindication for thrombotic procedures, were excluded from this analysis.

DATA ANALYSIS AND ETHICAL ASPECTS

The collected data were processed using Statistics Program for Social Sciences (SPSS) version 21. The following descriptive measures were used: central tendency and dispersion, mean and median, chi-square, and calculation of single and relative frequencies. The level of significance was $P<0.05$. The research was approved by the Research Ethics Committee involving individuals at Hospital Geral Roberto Santos, registered under number 2.993.032/2018.

RESULTS

During the period from January 1, 2016 to December 31, 2018, 540 individuals were included in the research and 224 patients were excluded. From the total number assessed, 192 (60.7%) presented post-thrombolysis reperfusion of the I-CVA, 124 (39.2%) were not reperfused, and 12 (2.2%) did not present data consistent with reperfusion.

The analysis of the data prior to the thrombolytic procedure and after being afflicted by acute I-CVA revealed that 316 individuals met the criteria for participating in the research. After the treatment of the collected data, it was evidenced that there was a predominance of the female gender, with stratified ages between 60 and 79 years, corresponding to 47.7% (83 women) of the total number of individuals for this age group and 51.8% of the total of 160 women in the research. Described under Table 1 is

Table 1. Description of the distribution of comorbidities by age of the reperfused individuals.

Comorbidities	Stratified age groups											
	27–59 years				60–79 years				80 years			
	Reperfused patients				Reperfused patients				Reperfused patients			
	Yes (119)		No (49)		Yes (176)		No (104)		Yes (3)		No (12)	
	n	%	N	%	n	%	n	%	n	%	n	%
Systemic arterial hypertension	57	68.7	32	66.7	73	68.9	52	76.5	2	66.7	8	100.0
Diabetes mellitus	19	22.9	5	10.4	34	32.1	23	33.8	0	0.0	3	37.5
Tobacco use at present or in the past	19	22.9	3	6.3	21	19.8	12	17.6	0	0.0	1	12.5
Alcohol intake at present or in the past	10	12.0	1	2.1	21	19.8	10	14.7	0	0.0	0	0.0
Cardiopathy	9	10.8	5	10.4	13	12.3	4	5.9	1	33.3	0	0.0
High cholesterol	1	1.2	1	2.1	6	5.7	1	1.5	0	0.0	0	0.0
Pulmonary arterial hypertension	0	0.0	1	2.1	0	0.0	0	0.0	0	0.0	0	0.0
Chagas disease	4	4.8	1	2.1	8	7.5	2	2.9	0	0.0	0	0.0

the age distribution of reperfused individuals by comorbidity in the studied population.

In relation to the post-thrombolysis outcome, around 40 patients (12.6%) died after 24 h of the procedure, mostly of the male gender (52.5%) and of the age group between 50 and 70 years. In total, 10.7% of the total thrombolized individuals relevant for the study were transferred to the intensive care unit, and 78.9% were discharged from the CVA unit of reference to the unit of low complexity (wards or open units) and/or sent home.

The study revealed that the studied patients, whether reperfused or not, had different frequencies of comorbidities and cardiovascular risk factors, characterizing the studied population as heterogeneous and of high cardiovascular risk.

With reference to individuals submitted to the reperfusion procedure, the average age was 60.5 years, and for non-reperfused individuals, it was of 62.8 years. The data of this study reveal a predominance of the female gender in the group of reperfused patients and the male gender in the non-reperfused group; both groups maintain moderate severity in the National Institutes of Health Stroke Scale (NIHSS) of hospital discharge and admission without statistical significance (p>0.18). Moreover, the average score of severity was 13.2 for the group of reperfused patients and 14.2 for non-reperfused patients, and the mean ejection fraction of both groups was within normal functionality, with mean values of 0.50 for reperfused patients and 0.62 for non-reperfused patients, as outlined in Table 2.

Table 2. Reperfused patients and some stratified clinical parameters.

Parameters		Reperfused										
		Yes					No					p-value
		n	%	Mean	95%CI		n	%	Mean	95%CI		
					Inferior	Superior				Inferior	Superior	
Death	Yes	14	7.3				26	21.0				<0.001
	No	178	92.7				98	79.0				
Stratified age	27–59 years	83	43.2				48	38.7				0.40
	60–69 years	58	30.2				38	30.6				
	70 years and over	51	26.6				38	30.6				
Gender	Male	89	46.4				67	54.0				0.18
	Female	103	53.6				57	46.0				
NIHSS admission	No stroke symptoms	0	0.0				0	0.0				0.04
	Minor stroke	2	1.3				6	5.8				
	Moderate stroke	98	64.5				51	49.5				
	Moderate-to-severe stroke	32	21.1				30	29.1				
	Severe stroke	20	13.2				16	15.5				
NIHSS discharge	No stroke symptoms	4	2.6				0	0.0				<0,01
	Minor stroke	35	22.9				23	22.1				
	Moderate stroke	81	52.9				40	38.5				
	Moderate-to-severe stroke	23	15.0				23	22.1				
	Severe stroke	10	6.5				18	17.3				
Dysfunction of the left ventricle	Yes	45	23.4				32	25.8				
	No	147	76.6				92	74.2				
Age				60.5	58.8	62.3			62.8	60.7	64.9	
Pumping force of the blood				0.59	0.56	0.61			0.62	0.59	0.65	
Severity scale upon hospital admission				13.2	12.3	14.0			14.1	12.9	15.3	
Severity scale upon hospital discharge				7.47	6.30	8.64			11.36	9.68	13.04	

The effectiveness of the applicability of the NIHSS on hospital admission and discharge is possible to observe in the data analysis. The categorization in intervals is considered, as per the previously described Table 1, and the results obtained are compared. Hospital admission and discharge NIHSS had minimum scores of 1 and maximum scores of 29 points. A higher score was observed for patients in the age group of 60–79 years, with an average score of 14.1 and a mean value of 14, above the younger age groups, where the average reached 12.7 for individuals under the age of 59 years and a mean value of 12 with a p-value of 0.04, described in Table 3.

There was a predominance in the NIHSS admission interval of 5 to 15 with approximately 58.4% (149 individuals), corresponding to patients in the symptom group with moderate sequelae, followed by moderate to severe levels of NIHSS admission 16–20 with 28.7% (62 individuals). To have a more reliable record of hospital discharge registers and a better understanding of the clinical status of patients, the NIHSS upon discharge was observed. These results indicate that approximately 79.7% (138 individuals) of the total NIHSS hospital discharge had scores of 1 to 15 points, considered light to moderate CVA, suggesting symptoms of minor to moderate intensity, as represented in Table 2. A loss of 19.3% (61 individuals) should be observed in the NIHSS admission data and 44.3% (140 individuals) of NIHSS discharge due to the fact that these were not registered in the physical records.

DISCUSSION

An association was observed between the I-CVA and prior cardiopathies and echocardiographic alterations with alteration to the left atrium and low ejection fraction common remodeling pathways due to an increase in cardiac cavities in advanced cardiopathies <40%. Effectively, various studies confirm the

Table 3. Distribution of the initial National Institutes of Health Stroke Scale and discharge of the studied population.

	Scores				% in stratified age			
	Stratified age			Total	Stratified age			Total
	27–59 years	60–79 years	80 years		27–59 years	60–79 years	80 years	
NIHSS admission								
Minor stroke	5	2	1	8	4.5	1.5	14.3	3.1
Moderate stroke	74	73	2	149	66.1	53.7	28.6	58.4
Moderate-to-severe stroke	22	39	1	62	19.6	28.7	14.3	24.3
Severe stroke	11	22	3	36	9.8	16.2	42.9	14.1
Total	112	136	7	255	100	100	100	100
NIHSS discharge								
No stroke Symptoms	3	1	0	4	2.7	0.7	0.0	1.6
Minor stroke	31	26	1	58	27.7	18.8	14.3	22.6
Moderate stroke	53	66	2	121	47.3	47.8	28.6	47.1
Moderate-to-severe stroke	17	27	2	46	15.2	19.6	28.6	17.9
Severe stroke	8	18	2	28	7.1	13.0	28.6	10.9
Total	112	138	7	257	100	100	100	100

NIHSS		N	Average	95%CI		Percentage			p-value
				Inferior limit	Superior limit	25	Median	75	
Severity scale upon admission	27–59 years	112	12.7	11.7	13.7	8,0	12,0	17,0	0.04
	60–79 years	136	14.1	13.2	15.1	9.0	14.0	18.0	
	80 years	7	16.6	9.5	23.6	15.0	18.0	22.0	
Severity scale upon discharge	27–59 years	77	8.3	6.7	9.8	3.0	6.0	14.0	0,31
	60–79 years	94	9.9	8.5	11.2	4.0	8.0	14.3	
	80 years	5	10.6	–0.4	21.6	3.5	6.0	20.0	

greater individual association of CVA in patients with reduced EF (<40%).

Our results corroborate venous thrombolysis as therapy for reperfusion in patients with I-CVA, reducing hospital morbimortality. Cardiovascular risk factors, prior cardiopathy, and advanced age are predominant in the studied population. These data reinforce the fact that with advanced age there is an increase in the risk for CVA, different from the younger age groups, where hereditary conditions, such as malformation, thrombophilia, and the use of medications and illicit drugs, may exist[8]. Despite the recognized relationship between age, cerebral Ischemia, and female gender for greater life expectation in this group, no positive association was found in this study.

Venous thrombolysis for arterial reperfusion is the main treatment behavior at present for ischemic CVA. Our results demonstrate the significant difference in mortality in patients submitted to the intervention. Our data on mortality and ischemic CVA are not correlated to the presence of systemic arterial hypertension (SAH) and diabetes, possibly due to the high level of comorbidities in the groups, CVA being a common complication of DM, both in young insulin-dependent individuals as in the elderly with type 2 DM. DM favors atherosclerotic disease of small and large vessels in the brain[9], and hyperglycemia occurs in over half of patients in the acute phase of the CVA, even those with a prior background of normoglycemia, and is associated with increased morbidity and mortality, independently of the age group, ischemic CVA mechanism, or extension of the ischemic lesion[10].

With respect to the CVA outcome, in this study, among the various risk factors studied, the relationship with alcohol intake was outlined. Alcohol intake is also associated with increased cardiopathy risks, such as systemic hypertension, atrial fibrillation, and dilated cardiomyopathy[10]. These conditions are juxtaposed, increasing even further the risk of CVA for alcohol-user patients. The low number of deaths, as well as the high number of discharges from the CVA sector to low-complexity units, suggests a trend for reference centers and early treatment in acute I-CVA, a trained team, and the quality of the service rendered by the institution.

Embolism of a cardiac origin is responsible for around 20 to 30% of all CVAs, and atrial fibrillation (AF) is the main cardiogenic cause[11]. AF increases between five and eight times the risk of CVA, being a significant risk factor for I-CVA; accordingly, it must be duly approached since these patients could benefit from prophylactic anticoagulation[12]. The findings of the study revealed that, among the prior factors and risks, AF appeared in only 8.2% (26 individuals). Of these, 61.5% (16 individuals) of the individuals with a successful outcome in venous reperfusion belonged to the female group, 76.9% had risk factors such as hypertension, and all of them were previously diagnosed with coronary diseases.

When associated with reperfusion and the severity score upon admission and discharge, most of the reperfused patients had moderate scores, suggesting that reperfusion had a low impact on the severity of the condition. Early treatment with a thrombolytic agent acts to dissolve the clots that obstruct the artery before irreversible tissue damage occurs and is the most indicated treatment for acute I-CVA.

There is strong evidence in the literature on the effectiveness of intravenous rtPA in reducing neurological damage[13]. The most important study assessing the role of thrombolytics in the I-CVA revealed improvement in at least four NIHSS scores after 24 h of the beginning of the symptoms. The favorable results of this study triggered the approval of the thrombolytic agent Alteplase (rtPA) in June 1996. However, there is some controversy about the use of the medication, especially in more extensive CVAs, for patients of advanced age and when there is a delay in the treatment[14].

CONCLUSION

An association was found between successful reperfusion by venous chemical thrombolysis and lower mortality in patients with acute I-CVA. Risk classifications predict favorable results for arterial reperfusion. The presence of cardiopathies with reduced ejection fraction in the echocardiogram was significant and associated with the severity of the I-CVA in the studied population. New studies could better establish these variables and their correlation with severity.

AUTHORS' CONTRIBUTIONS

ARO: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **PAPJ:** Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization. **FVB:** Formal Analysis, Supervision. **EMN:** Formal Analysis, Methodology, Supervision, Validation, Visualization. **JOF:** Data curation, Formal Analysis, Methodology, Project administration, Supervision, Validation, Visualization. **LR:** Supervision, Validation, Visualization. **ACIC:** Formal Analysis, Supervision, Validation, Visualization, Writing – review & editing. **RA:** Formal Analysis, Methodology, Project administration, Supervision, Validation, Visualization.

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ORIGINAL ARTICLE

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Updated threshold, renewed problems: should the diagnostic criteria of polycythemia vera be reconsidered? A retrospective cross-sectional cohort study

Anıl Uçan[1*], Müfide Okay Özgeyik[2]

SUMMARY

OBJECTIVE: This aim of this study was to evaluate hemoglobin and hematocrit values of polycythemia vera and secondary polycythemia patients with updated World Health Organization thresholds. In addition, by determining our own threshold values, we aimed to demonstrate the necessity of bone marrow biopsy and genetic analysis to be used for further diagnosis in patients with high-normal hematocrit and hemoglobin values.

METHODS: A cross-sectional and retrospective study was performed with the medical records of patients from Eskisehir City Hospital hematology clinics and outpatient clinics between July 1, 2019 and July 1, 2020. The study included patients with polycythemia, divided into two groups according to polycythemia vera and secondary polycythemia. A bone marrow biopsy was performed on patients with either Janus kinase mutation positivity and/or subnormal erythropoietin levels. Receiver operating characteristics analysis was used to find threshold values, and the diagnostic efficiency of these values in differentiating World Health Organization thresholds in 2008 and 2016 was evaluated.

RESULTS: A total of 73 patients were included. The median age was 43.5 years (min: 18; max: 84). The hematocrit value of 54.1 was predicted to diagnose polycythemia vera with a sensitivity of 45% and a specificity of 80%. Subsequent analysis revealed that an hemoglobin value of 17.7 was indicative of diagnosing polycythemia vera with a sensitivity of 60% and a specificity of 63%. The mean follow-up length was 6.4 months (2–12).

CONCLUSION: Our study demonstrated that modified World Health Organization criteria might lead to unnecessary additional tests for polycythemia vera patients with high-normal hemoglobin and hematocrit values.

KEYWORDS: Polycythemia. Diagnosis. Hematologic tests. Hematocrit. Hemoglobins.

INTRODUCTION

Polycythemia vera (PV) is a member of the Philadelphia chromosome-negative (Ph-) chronic myeloproliferative neoplasm diseases[1]. The incidence of the disease is 0.01–2.61 per 100,000 people per year, and the mean age at diagnosis is 60 years[1,2]. Like all myeloproliferative neoplasms (MPNs), PV could transform into acute or chronic myeloid-based myeloid leukemia or neoplasms (yearly incidence of transformation: 0.38% for PV, 0.37% for ET)[3,4]. Secondary acute myeloid leukemias (sAML) were associated with an increased risk of high mortality and a worse prognosis[3,5]. Therefore, the diagnosis of PV plays a critical role in maintaining disease mortality. The current treatment goals in PV are to prevent thromboembolic complications and relieve symptoms. While achieving these goals definitely improves quality of life and survival rates, the existing medical therapies cannot stop PV from developing into leukemia or neoplasms.

However, the condition that should be excluded in the differential diagnosis at the very beginning of the diagnostic algorithm is the presence of secondary polycythemia (SP). It is observed that SP is often caused by tissue hypoxia and rarely by neoplasms that secrete erythropoietin (EPO)[6,7], and is frequently associated with smoking and chronic obstructive pulmonary disease (COPD). It should be highlighted that, unlike primary polycythemia, the erythroid progenitor lineage of cells does not intrinsically exhibit a deficiency. In both cases of PV and SP, patients usually have nonspecific symptoms on presentation, including fatigue, headache, and dizziness. These similarities have become an obstacle for clinicians to overcome in differential diagnosis for years, and appropriate and cost-effective diagnostic criteria have been tried to be established.

A classification containing updates in the diagnosis of the disease was published by the World Health Organization (WHO) in 2016[8], and criteria are nowadays frequently used in the diagnosis of MPNs. In the 2016 classification, an update was made, and the first major criteria of high hemoglobin (Hb) (>16.5 g/dL in men or >16.0 g/dL in women) levels were decreased

[1]Eskisehir City Hospital, Department of Internal Medicine – Eskişehir, Turkey.
[2]Eskisehir City Hospital, Department of Hematology – Eskişehir, Turkey.
*Corresponding author: anil.ucan@saglik.gov.tr

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compared to 2008 hematocrit (Hct) (>18.5 g/dL in men or >16.5 g/dL in women)[8]. The suggested revised threshold limits for Hb and Hct, especially for men (men: 13.5–17.5 g/L and Hct: 38.8–50%; women: 12.0–15.5 g/L and Hct: 34.9–44.5%), exhibit a remarkable match with the common reference values found in healthy individuals[1,9]. These patients may be overdiagnosed with PV and examined for serum erythropoietin (EPO) levels and/or Janus kinase 2 (JAK2) mutations without necessity.

As these new thresholds are updated with data from clinico-pathological databases of patients, they have led clinicians to suspect that the impact of the thresholds on disease diagnosis may not be widely applicable in the general population with demographic and geographical differences. The debate continues about the strategies for the overdiagnosis of Philadelphia chromosome-negative (Ph-) chronic MPNs.

In this study, we aimed to compare the Hb and Hct levels in PV and SP patients to current WHO standards. Furthermore, by establishing our own threshold values, we wanted to investigate the necessity of bone marrow biopsy and genetic analysis for further diagnosis in patients with high-normal Hct and Hb values.

METHODS

Study design and ethical considerations

This cross-sectional retrospective study includes the evaluation of randomly selected 200 patients who applied to Eskisehir City Hospital hematology clinics and outpatient clinics between July 1, 2019 and July 1, 2020. Approval for the study was obtained from the local ethics committee of Eskişehir Osmangazi University (approval number 2020/328) and was carried out in accordance with the Declaration of Helsinki principles and all applicable regulations. Informed consent was waived (a retrospective study).

Study groups and eligibility criteria

A total of 458 patients with PV and SP were identified. A flowchart of the study patients is shown in Figure 1. However, out of these subjects, an adequate bone marrow biopsy and complete medical record were available in 85 patients with PV and 115 patients with SP. Patients with PV were diagnosed according to the WHO criteria by two independent hematology specialists[8,10]. However, 11 patients who did not match the WHO criteria for PV at the point of diagnosis or had a mixed diagnosis of myelodysplasia or other myeloproliferative neoplasms such as essential thrombocytosis or primary myelofibrosis were excluded.

The inclusion criteria were being 18 years of age or older and being referred to hematology outpatient clinics with symptoms of polycythemia and hyperviscosity such as erythromelalgia and pruritus. A total of 12 patients diagnosed with essential thrombocythemia were excluded from the study. In patients with no or inadequate data, being pregnant, being under 18 years of age, and using drugs that may cause polycythemia were excluded.

Data collection

Patient baseline characteristics, comorbidities, smoking status, clinical findings, laboratory results (Hb, Hct, white blood cell

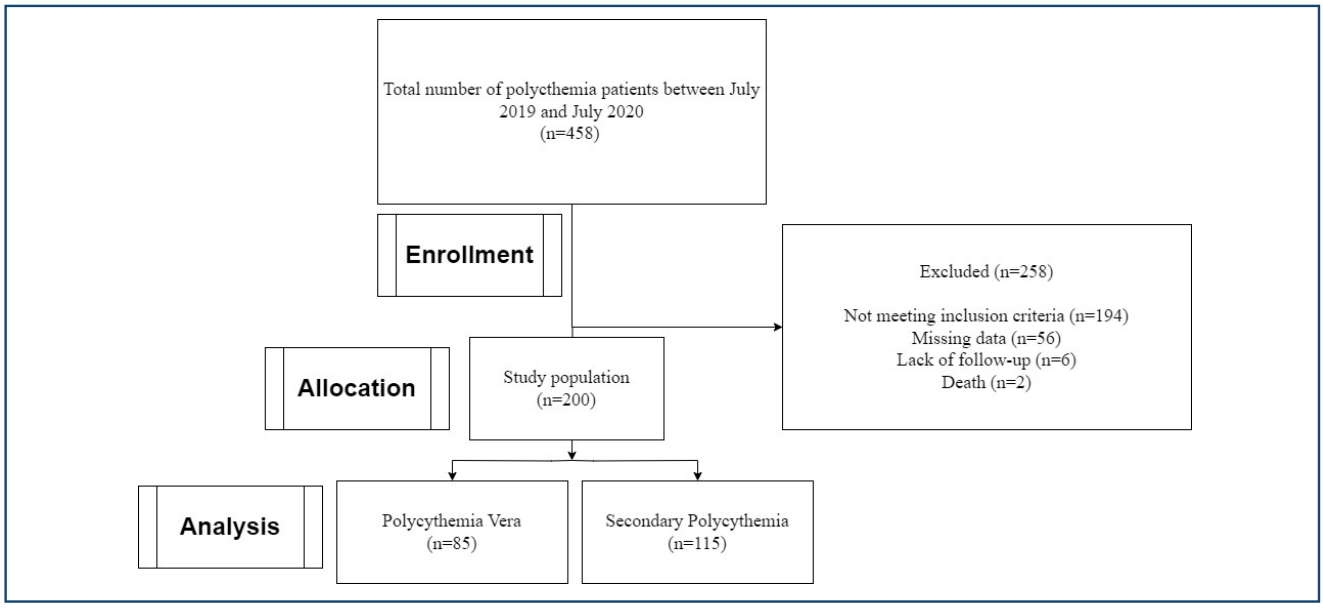

Figure 1. Flowchart of the study.

(WBC), neutrophil count (Neu), platelet count (Plt), lactate dehydrogenase (LDH), and serum EPO levels), and bone marrow results were obtained from the medical record archives. As a result, we also excluded 56 patients with insufficient data. The mean follow-up length was 6.4 (2–12) months. Six patients who received initial therapy at another hospital were excluded due to a lack of follow-up at our hospital. Two patients were lost to follow-up. In total, follow-up data were available for 200 patients.

All bone marrow biopsies are examined at the Department of Pathology, Eskişehir City Hospital. When all hematologic parameters were within the defined delta limits and no suspected red flags were identified, the CBC was considered normal as follows: WBC: 3–10×10^3/μL (neutrophils: 1–10×10^3/μL; platelets: 150–450×10^3/μL); and no band neutrophil, metamyelocyte, myelocyte, promyelocyte, blast cell, or plasma cells were detectable. The samples were run on a Cell-Dyn Ruby® (Abbott, United States) automated hematology analyzer.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) was used to conduct the statistical analysis (SPSS, version 22.0, SPSS Inc., Chicago, IL, USA). The distribution of the data was analyzed using the Kolmogorov-Smirnov method. Non-normally distributed data were expressed as a median, interquartile range (IQR), and range. All the continuous data included in this study had a non-normal distribution. Chi-square analysis was used for the comparison of frequencies of the major manifestations and the Student's t-test (Mann-Whitney U test) for the comparison of median values between groups. The ROC analysis was used to determine the threshold values of the numerical parameters that were used to predict disease status and to evaluate the indicators' accuracy. Youden's index was used for selecting the cutoff value. The area under the curve (AUC) was used as an estimation of diagnostic accuracy. In addition, sensitivity and specificity for Hb and Hct were obtained. Missing data from patients that were more than 50% were not included in the study. According to the analysis results, a p-value of 0.05 or below was accepted as statistically significant. For the sake of accuracy, the p-values are given with four digits following the decimal point.

RESULTS

Comparing baseline characteristics

Sociodemographic and clinical profiles and comparisons between groups are shown in Table 1.

Laboratory findings and receiver operating characteristic analysis

Further statistical tests revealed significant differences between Hb and Hct results among the two groups in our study. If we now turn to other laboratory values (WBC, Neu, Plt, and LDH), they were also significantly higher in PV compared to the SP arm except for EPO (Table 2).

The distribution of Hb and Hct values of the patients in the study groups was recorded. The Hct value of 54.1% was predicted to diagnose PV with a sensitivity of 45% and a specificity of 80%. The area under the ROC curve (AUC) was 0.655 (95%CI 0.585–0.721) (Figure 2).

Table 1. Sociodemographic and clinical characteristics of the patients diagnosed with polycythemia vera and secondary polycythemia.

	PV (n=85)	SP (n=115)	p-value
Age (years), median (IQR)	43 (18–84)	44 (18–79)	0.598*
Male sex—n (%)	75 (88.2)	106 (92.2)	0.348**
Smoking—n (%)	27 (31.8)	57 (49.6)	**0.012****
Comorbid diseases			
Diabetes (%)	10.6	21.7	**0.038****
Hypertension (%)	21.2	23.5	0.701**
Dyslipidemia (%)	14.1	14.8	0.895**
CAD (%)	5.9	7.8	0.595**
Hyperviscosity symptoms—(%)	45.9	22.6	**0.001****
Eritromelalgia (%)	17.6	6.1	**0.010****
Pruritis (%)	18.8	5.2	**0.002****

Data are expressed as the median (IQR) or n (%). IQR: interquartile range; n: sample size; CAD: coronary artery disease; *Mann-Whitney U test; and **χ2 test. p<0.05 (in bold) indicates statistical significance.

Further analysis showed that an Hb value of 17.7 predicted the diagnosis of PV with a sensitivity of 60% and a specificity of 63%. The AUC was 0.621 (95%CI 0.550–0.689) (Figure 3).

DISCUSSION

This study defined the factors related to the use of updated laboratory thresholds in 2016 and evaluated patients with polycythemia symptoms admitted to the hematology outpatient clinic. The present analysis was performed for two purposes. First, the study was to define the evaluation of PV and SP patients between the updated and old thresholds of Hb and HCT values[1]. Second, the study aimed to investigate the necessity of a bone marrow biopsy and genetic analysis to be used in further diagnosis.

The first focus point of discussion on the updated diagnostic criteria in 2016 is the lowering cutoff value in Hct and Hb for a major criterion[11]. A strong relationship between Hct and Hb levels and PV diagnosis has been debated in the literature[8,12-15]. In a study, it was suggested that bone marrow plays an important role in strengthening the relationship between Hb and Hct values and diagnosis, which can also help distinguish PV from other MPNs[16]. In a large-scale retrospective study based on these and similar concerns, 2016 WHO diagnostic criteria were strictly applied and performed on randomly selected people in the Canadian and Brazilian populations. The study was successfully predicted that the annual PV incidence would increase 12 times for men and 3 times for women when using 2016 criteria[17]. This study also suggested that standardizing diagnostic approaches for MPNs across the country could contribute to cost-effectiveness and avoid unnecessary further research. Barbui et al., conducted a study to investigate the usefulness of independent use as a major diagnostic criterion. The study also suggested that the Hb/Hct levels may lead to a significant increase in excessive diagnostic tests, such as EPO serum levels,

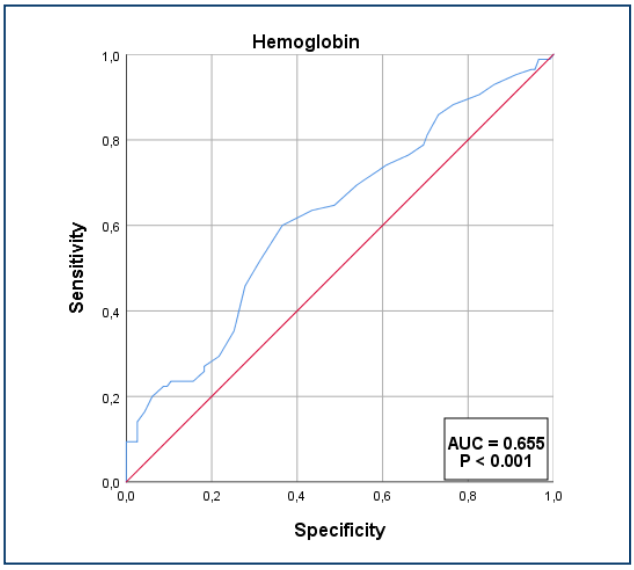

Figure 2. Receiver operating characteristic curve for the hemoglobin.

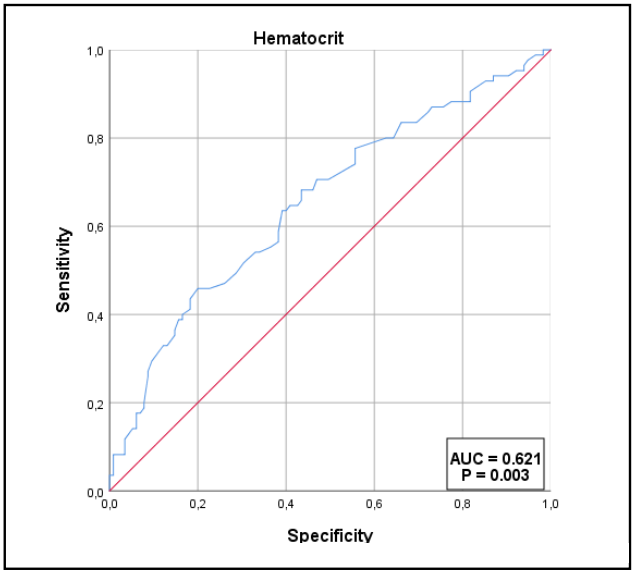

Figure 3. Receiver operating characteristic curve for the hematocrit.

Table 2. Comparison of laboratory parameters between polycythemia vera and secondary polycythemia patients.

	PV (n=85)	SP (n=115)	p-value
Hb (g/dL)	17.9 (16.0–21.7)	17.4 (16.0–19.2)	**0.032***
Hct (%)	54.1 (48.4–65.3)	52.0 (48.0–61.7)	**0.001***
WBC (10^3/µL)	8.4 (3.7–47.3)	7.4 (3.0–15.4)	**<0.001***
LDH (mg/dL)	220 (139–1358)	209 (146–436)	**<0.001***
Neutrophil (10^3/µL)	5.3 (1.7–35.0)	4.3 (1.6–13.4)	**<0.001***
Platelets (10^3/µL)	271 (122–993)	241 (136–637)	**<0.001***
Erythropoietin (IU/L)	6 (1–12)	9 (4–42)	**<0.001***

All data are presented as the median (IQR); IQR: interquartile range; n: sample size; Hb: hemoglobin; Hct: hematocrit; WBC: white blood cell; LDH: lactate dehydrogenase; and *Mann-Whitney U test. p<0.05 (in bold) indicates statistical significance.

molecular investigation (JAK2V617 and exon 12), and biopsies of the bone marrow[12]. It is also important to consider that 0.1% of all bone marrow biopsy interventions could cause serious side effects, including infection, bleeding, and even death[12,14].

The most crucial aspect of the updated criteria in 2016 was to identify masked and clinically suspected cases[10,12]. Changing Hct and Hb thresholds in 2016 made some changes and was shown in a study to cause a 36% increase in the diagnosis of PV over the 2008 version[11]. In a study in which 2,056 suspected MPN patients were evaluated, 132 patients were diagnosed with the WHO 2008 criteria, while 154 patients were diagnosed with the WHO 2016 criteria[10]. The findings made an important contribution to the treatment of the patients who did not fully meet the diagnostic criteria. In contrast to earlier findings, when we considered the Hb and Hct thresholds of the updated criteria, none of the patients were diagnosed as PV instead of SP. One unanticipated finding was that the cutoff values found as 17.7 for Hb and 54.1 for Hct in the ROC analysis are above the current guideline[12]. A possible explanation for this might be geographic and demographic differences.

Another question in this study sought to determine the use of other complete blood count parameters for differential diagnosis. WBC, neutrophil, platelets, and LDH tests among the PV and SP groups were significantly different ($p<0.001$). This finding was also reported by Sandes et al., as mentioned in leukocytosis and thrombocytosis, which could be useful in PV diagnosis[14]. In cases with borderline levels of Hb and Hct, using other achievable laboratory parameters in combination for diagnosis may benefit researchers[12].

Further statistical tests in the study revealed the prevalence of polycythemia was significantly higher in males compared to female patients. However, no significant difference between the two groups in gender was evident ($p=0.348$). These results are supported by many recent studies. In 426 PV cases followed between 2001 and 2011, the incidence rate was found to be lower in the female gender, especially with advanced age[18]. In contrast, in a systematic meta-analysis, the incidence of MPNs did not differ significantly between men and women[19]. Therefore, it should be noted that the updated diagnostic criteria for polycythemia should take into account the fundamental differences in hemoglobin and hematocrit between men and women, and gender differences observed in secondary polycythemia introduce the risk.

LIMITATIONS

The limited number of patients and the low diagnostic efficacy of Hb and Hct values in this study can be counted among the limitations of the study. The study also partially illustrates the usefulness of ROC curve analysis applied to a common clinical laboratory test and how it can help in defining an appropriate range of values and a specific cutoff point for a particular population. Although the current study is based on a small sample of participants in PV, for the first time to the best of our knowledge, it adds to our understanding of the impact of the revised diagnostic criteria in 2016.

CONCLUSION

As shown in our study, the updated criteria in the WHO classification applied to PV patients for differential diagnosis with high-normal Hb and Hct levels could cause unnecessary further investigation. Since Hb and Hct values used in the diagnostic criteria cannot be generalized, we think that a good clinical evaluation will prevent unnecessary investigations from being ordered in the diagnosis of PV. Differences in demographics and geography may force us to reassess the diagnostic criteria. A significant rise in needless tests for diagnosis, such as EPO serum dose, molecular testing (JAK2V617 and exon 12), and bone marrow biopsies, may result from the isolated use of the suggested Hb/Hct levels as a description of polycythemia. In spite of significant updates, we still need to support our findings with larger, prospective, and randomized studies to increase the diagnostic precision of MPNs.

AVAILABILITY OF DATA AND MATERIALS

The data are available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

AU: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MOÖ:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of oral isotretinoin treatment for acne vulgaris patients on anger responses and the relationship with temperament

Esra Yancar Demir[1]*, Özlem Karadağ Köse[2]

SUMMARY

OBJECTIVE: Isotretinoin is the only medication against all the factors involved in acne vulgaris pathogenesis. The aim of our study was to verify whether patients with acne vulgaris receiving isotretinoin therapy exhibit elevated anger levels and to observe the correlation between age, temperament traits, and anger.

METHODS: The study group comprised a sum of 100 cases, involving 50 individuals with acne vulgaris-required high-dose retinol therapy and 50 controls who did not start any medication.

RESULTS: Our study showed that anger levels increased with drug use. A positive correlation between cyclothymic temperament, the anxiety-related behavior subdimension, and the introvert and passive-aggressive subdimension of interpersonal anger reactions has been recognized. In addition, a positive one was observed between hyperthymic temperament and the introvert subdimension, which is one of the anger-related thoughts and interpersonal anger reactions.

CONCLUSION: This study elucidates anger dimensions such as anger-related thoughts, behaviors, and reactions in individuals who received retinol treatment for acne vulgaris. In addition to anger and its dimensions, temperament was also investigated. Although several studies have investigated the relationship between acne vulgaris and psychiatric symptoms, to the best of our knowledge, no research has been reported in the English-language literature regarding the relationship between anger dimensions and temperament after retinol treatment that might make our study an original and valuable contribution to the literature.

KEYWORDS: Acne vulgaris. Anger. Isotretinoin. Aggression. Side effects. Temperament.

INTRODUCTION

Acne vulgaris (AV), *per se*, is a disease characterized by inflammatory changes in the pilosebaceous follicles occurring in the form of comedones, papules, pustules, nodules, and cysts that may cause appearance disorders due to serious permanent scarring. Generally, the disease is defined to affect adolescents and young adults with different clinical appearances, and the severity of the disease and the individual's perceptions may be different[1]. There are at least three types of interaction proposed between AV and mental factors: (1) there is a complicated relationship between stress and AV involving the neuroimmune cutaneous system and hypothalamic-pituitary axis with AV observed or becoming more severe via this pathway; (2) AV develops secondarily in patients with psychiatric symptoms such as anxiety, depression, social phobia, and low self-esteem; and (3) just as in body dysmorphic disorder, a primary psychiatric disorder is the focus of acne[2]. *De facto*, isotretinoin is the only medication affecting all factors playing a role in the pathogenesis of acne[3], and its correlation with depression, anxiety, and anger control is still a controversial issue. The high incidence of depression in society makes it difficult to distinguish depression triggered by other causes like isotretinoin. When the literature studying depression as a side effect of isotretinoin is examined, in general, there is no common point with studies proposing that isotretinoin causes depression mainly in the form of case reports and limited controlled studies supporting the correlation between isotretinoin and depression. Contrarily, many retrospective and prospective controlled studies conclude no significant correlation between isotretinoin and depression. However, cases developing depressive symptoms during isotretinoin therapy exhibit that patients need to be closely monitored for the development of anxiety and depression during the treatment process[4]. Some authors state that acne should be dealt with as an organic event with personality traits having no effect on the development of acne[5], while some identify the disrupted quality of life[6], high anger levels[7], and difficulty in terms of social and functioning[1] among acne cases.

This study purposed first to test whether there is an increase in anger levels at the scale level observed in clinical practice

[1]Boylam Psychiatric Hospital – Ankara, Turkey.
[2]Department of Dermatology and Venereology – İstanbul, Turkey.
*Corresponding author: edemiryancar@gmail.com
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among AV cases treated with isotretinoin and second to observe the correlation between temperament traits and anger. In this way, we aimed to draw more attention to the psychological effects of isotretinoin, which is frequently used in AV by dermatologists in clinical practice, and to raise awareness about the initiation of treatment in patients at risk in cooperation with psychiatrists.

METHODS

Ethical aspects

This study was conducted according to the Declaration of Helsinki and approved by the Clinical Research and Ethics Committee linked to Ordu University (No. #2014/01).

Study design

This study incorporated a sum of 100 cases, including a 50-person patient group admitted to the Dermatology Clinic due to AV and started high-dose isotretinoin and a 50-person control who had not possessed isotretinoin but only antibiotics and local acne medication between January 2017 and March 2018. The study examined the correlation between age, multidimensional anger levels, and temperament traits of patients attending our clinic due to AV grades 4–5 according to the Global Acne Severity Scale and beginning high-dose isotretinoin. In addition, whether there was a difference in multidimensional anger levels based on sex and educational status of participants had been investigated, along with the variations in multidimensional anger levels in the patient group in three different periods (i.e., initial, first month, and third month). Our exclusion criterion was that the patients had previously or currently received psychiatric treatment. This assessment was made on the basis of the patients' statements. As such, three diagnostic tools were used to collect the necessary information: (i) the Sociodemographic and Clinical Information Form, (ii) the Multidimensional Anger Scale (MAS), and (iii) the Temperament Scale (TEMPS-A). To this end, informed consent had been given to all patients, and their signatures were obtained.

Data collection tools

In this research, three tools were used to collect the necessary information:

1. Sociodemographic and Clinical Information Form
2. MAS
3. TEMPS A temperament scale

Sociodemographic and Clinical Information Form:

In accordance with the aim of the research, this tool was developed by the researchers to collect information about sociodemographic and clinical data.

MAS:

The scale was developed by Balkaya and Şahin[8] with the aim of determining feelings, thoughts, and attitudes about anger in individuals, and validity and reliability studies were completed. The MAS comprises 158 items inquiring about five different dimensions[8].

Temps-A Temperament Scale:

Being developed by Akiskal, this scale comprises 100 items to determine 5 temperaments[9], namely, depressive, cyclothymic, hyperthymic, irritable, and anxious. Individuals answer the questions with yes or no based on consideration of all experiences. The Turkish validity and reliability study was completed by Vahip et al[10].

Statistical analyses

The Cronbach's alpha internal consistency coefficient was calculated for reliability studies for answers given by patients with AV to the MAS. According to the Shapiro-Wilk test (for $n<50$) results, the points for the MAS as a whole and for each subdimension used in the research had a normal distribution in terms of error ($p>0.05$). *In fine*, differences between total points for scales and subdimensions in terms of the patient group and sex were determined with the Student's t-test, whereas differences in terms of educational status were determined with the one-way analysis of variance and the Tukey's multiple comparison test. Additionally, the correlation between patient age, multidimensional anger levels, and temperament traits was examined with the Pearson correlation coefficient.

RESULTS

The participants in the study were divided into two groups in the initial period based on whether or not they were taking retinoic acid. In terms of responses to the MAS, the points obtained for the thoughts related to the anger dimension (not taking retinoic acid=70.04; taking retinoic acid=79.74) were identified to be significantly different. Responses of participants to the anger scale for isotretinoin and control groups are summarized in Table 1. In the initial period, there were no significant differences identified in terms of responses to the MAS based on the gender and educational status of participants ($p>0.05$).

In terms of responses to the MAS in the third month, the patients taking retinoic acid had statistically significantly higher points for the dimension of thoughts related to anger and all

Table 1. Participant responses to multidimensional anger scale in the beginning and third month.

Beginning-Multidimensional Anger Scale	Isotretinoin (n=50)	Control (n=50)	p-value
Thoughts related to anger	79.74±17.68	70.04±12.04	0.002*
Anger-oriented thought	19.26±9.25	18.62±5.94	0.691
Angry thoughts toward others	20.66±7.81	19.82±6.22	0.553
Angry thoughts toward themselves	15.68±5.70	15.26±4.37	0.680
Angry thoughts toward the world	10.70±5.40	10.90±4.74	0.844
Behavior related to anger	69.16±14.76	70.04±12.04	0.745
Aggressive	27.20±8.66	25.60±8.03	0.340
Calm	29.78±8.60	31.50±6.77	0.269
Anxious	12.18±3.22	12.94±2.88	0.217
Interpersonal anger reactions	122.80±37.20	132.68±33.06	0.164
Revenge	55.28±22.11	58.58±20.95	0.445
Passive-Aggressive	31.64±9.18	33.18±8.40	0.542
Introversion	29.70±8.47	31.84±6.95	0.170
Unconcerned	8.18±3.21	9.08±3.57	0.188
Third month Multidimensional Anger Scale	**Isotretinoin (n=50)**	**Control (n=50)**	**p-value**
Thoughts related to anger	79.06±26.70	70.04±12.04	0.002*
Anger-oriented thought	24.04±8.72	18.62±5.94	0.001*
Angry thoughts toward others	23.78±8.22	19.82±6.22	0.008*
Angry thoughts toward themselves	18.36±6.14	15.26±4.37	0.004*
Angry thoughts toward the world	12.88±5.02	10.90±4.74	0.045*
Behavior related to anger	79.74±17.68	70.04±12.04	0.002*
Aggressive	35.40±9.39	25.60±8.03	0.001*
Calm	31.02±9.39	31.50±6.77	0.738
Anxious	13.32±3.11	12.94±2.88	0.528
Interpersonal anger	145.56±35.29	132.68±33.06	0.063

*p<0.005.

subdimensions (thoughts about anger, angry thoughts toward others, angry thoughts toward themselves, and anger thoughts toward the world), points for the revenge subdimension of the interpersonal anger reaction dimension, the behavior related to anger dimension, and the aggressive subdimension compared with patients not taking retinoic acid. The differences between responses to the MAS based on the gender of participants in the third month are given in Table 1. In terms of responses to the MAS in the third month, there were no statistically significant differences identified between male and female participants and on educational status in terms of all dimensions and subdimensions.

The variation over time of responses to the MAS initially and in the first and third months of patients taking retinoic acid is given in Table 3. There was a linear increase in points determined over time for all dimensions and subdimensions (apart from passive-aggressive and introversion) for responses of patients receiving retinoic acid to the MAS from initially to the first and the third months ($p<0.05$) (Table 2).

The correlation levels and significance between patients' age, multidimensional anger levels, and temperament traits are given in Table 3. According to correlation analysis, the thoughts about the anger subdimension of the MAS were positively and significantly correlated with depressive temperament, and hyperthymic temperament on the TEMPS scale. There were positive and significant correlations identified between depressive temperament and thoughts about anger, angry thoughts toward others, angry thoughts toward themselves, and angry thoughts toward the world. There were positive significant correlations between depressive temperament and the interpersonal anger reaction subdimension of anxious reaction, and the behavior related to the anger subdimension of introverted behavior. There were

Table 2. Comparison of patients responses to the multidimensional anger scale in the beginning and in the third month.

Multidimensional Anger Scale	Initial (n=50)	Third month (n=50)	p-value
Thoughts related to anger	79.74±17.68	79.06±26.70	0.001*
Anger-oriented thought	19.26±9.25	24.04±8.72	0.001*
Angry thoughts toward others	20.66±7.81	23.78±8.22	0.001*
Angry thoughts toward themselves	15.68±5.70	18.36±6.14	0.001*
Angry thoughts toward the world	10.70±5.40	12.88±5.02	0.001*
Behavior related to anger	69.16±14.76	79.74±17.68	0.001*
Aggressive	27.20±8.66	35.40±9.39	0.001*
Calm	29.78±8.60	31.02±7.49	0.009*
Anxious	12.18±3.22	12.26±3.32	0.014*
Interpersonal anger reactions	122.80±37.20	145.56±35.29	0.001*
Revenge	55.28±22.11	72.68±20.04	0.001*
Passive-Aggressive	29.64±9.15	31.72±8.71	0.084
Introversion	29.70±8.47	31.18±7.71	0.078
Unconcerned	8.18±3.21	9.98±2.23	0.001*

*p<0.005.

positive and significant correlations identified between cyclothymic temperament with anxious response, passive-aggressive, and introversion subdimensions. There were positive and significant correlations between hyperthymic temperament and angry thoughts toward others, angry thoughts toward themselves, angry thoughts toward the world, interpersonal anger reactions, and introverted behavior related to anger. For irritable temperament, there were positive significant correlations with revenge, related to anger, passive-aggressive, and introverted behavior subscales, and a negative significant correlation with the aggressive subscale of the interpersonal anger reactions. For anxious temperament, there was a positive significant correlation found with the revenge behavior related to the anger subscale.

DISCUSSION

Acne dermal lesions, *per se,* are a chronic inflammatory disease of the skin of *Homo sapiens* which might lead to low self-esteem, anxiety, depression, and stigmatism which might frequently impair the quality of life seriously[11-13]. Mental effects may give rise to AV which might increase the risk of anxiety, depression, and suicide[14]. Moreover, retinoic acid is a drug that is recommended for treating nodulocystic acne but is used in milder forms and affects more than one mechanism[15,16]. Anger has been revealed as a variable in many studies; however, as far as we are aware, no study that examines the subdimensions of anger, such as thoughts, behaviors, and reactions, that may develop in stages in patients with retinol therapy has been conducted. When patients with and without retinol administration were examined in the analyses performed, the anger-related thoughts (anger-related thoughts toward others, one's self, and the world) increased significantly in the measurements performed at the end of the third month compared with the first month. In the measurement performed during the first application, there was a significant increase in the anger-related thought dimension and in all subdimensions in the third month. While the anger response was in the form of passive-aggressive in the first month, it was in the form of revenge in the measurements in the third month. While the patients did not define any behaviors related to anger at the beginning, they stated that they presented their anger as aggression in the third month. Our study showed that anger levels increased with drug use. In another study that supported the results of our study, it was revealed that the irritability of the patients significantly increased at the end of the third month with the use of medication[15]. Moreover, meta-analysis studies showed a significant relationship between retinoic acid use and mood disorders[17,18]. However, there are studies that do not support that drug use causes an increase in psychiatric diseases[19]. Averil et al. demonstrated that men and women were similar in terms of anger styles[20], which is consistent with our study. However, some studies concluded that anger revealed a significant difference between men and women, that anger and aggressive behaviors were more common in men than women, and that men directly expressed their feelings of anger. Although some studies show that anger responses decrease with

Table 3. Correlations between multidimensional anger levels and temperament characteristics.

Dimension and subdimensions	DepressiveT	CycloT	HyperthymicT	IrrT	AnxiousT
Thoughts related to anger					
r-value	**0.307**	0.147	**0.220**	-0.022	0.111
P-value	**0.002**	0.146	**0.028**	0.829	0.271
Anger-oriented thought					
r-value	**0.254**	0.145	0.189	-0.073	0.098
P-value	**0.011**	0.150	0.060	0.473	0.330
Angry thoughts toward others					
r-value	**0.283**	0.108	**0.201**	-0.005	0.116
P-value	**0.004**	0.286	**0.045**	0.960	0.251
Angry thoughts toward themselves					
r-value	**0.254**	0.070	**0.204**	-0.062	0.068
P-value	**0.011**	0.491	**0.041**	0.542	0.500
Angry thoughts toward the world					
r-value	**0.348**	0.228	**0.221**	0.087	0.122
P-value	**0.000**	0.022	**0.027**	0.389	0.226
Interpersonal anger reaction					
r-value	0.120	0.024	**0.250**	-0.109	0.036
P-value	0.234	0.816	**0.012**	0.278	0.723
Aggressive					
r-value	0.017	-0.037	0.284	**-0.260**	-0.024
P-value	0.869	0.713	0.004	**0.009**	0.815
Calm					
r-value	0.147	0.010	0.102	0.078	0.075
P-value	0.145	0.921	0.311	0.439	0.459
Anxious					
r-value	**0.231**	**0.226**	0.132	0.102	0.091
P-value	**0.021**	**0.024**	0.191	0.312	0.370
Behavior related to anger					
r-value	0.179	0.276	0.252	0.106	0.035
P-value	0.074	0.005	0.012	0.295	0.731
Revenge					
r-value	0.174	0.150	0.190	**0.440**	**0.205**
P-value	0.076	0.129	0.058	**<0.001**	**0.042**
Passive-Aggressive					
r-value	0.116	**0.348**	0.158	**0.235**	0.165
P-value	0.250	**<0.001**	0.116	**0.018**	0.102
Introversion					
r-value	**0.284**	**0.317**	**0.209**	**0.270**	0.086
P-value	**0.004**	**0.001**	**0.037**	**0.007**	0.395
Unconcerned					
r-value	0.134	-0.034	0.146	0.005	0.004
P-value	0.185	0.735	0.146	0.964	0.972

r are the correlation coefficients between examined traits; P-value are significant values.

age, some report that there is no difference in terms of anger symptoms. This study did not reveal a significant correlation between age and dimensions of anger. The results of our study support the positive correlation between depressive temperament and all subdimensions of anger-related thoughts, the anxiety behavior subdimension of anger-related thoughts, and the introvert subdimension of interpersonal reactions. Our study supports the positive correlation between cyclothymic temperament and the anxiety-related behavior subdimension, and the introvert and passive-aggressive subdimension of interpersonal anger reactions. Individuals with hyperthymic temperament, however, draw a profile that is friendly, extroverted, overly talkative, self-confident, optimistic, and well-planned. When the relationship between hyperthymic temperament and anger is examined, our study supports the positive correlation between hyperthymic temperament and the introvert subdimension, which is one of the anger-related thoughts and interpersonal anger reactions. This result can be thought of as a reaction that is inconsistent with the hyperthymic temperament trait. However, due to their overly optimistic nature, they may be using the rationalization defense mechanism a lot and may not have an open reaction which can be considered a reaction that is inconsistent with the hyperthymic temperament trait[8,17,21]. Our study supports the positive correlation between irritable temperament and revenge, passive-aggressive, and introvert subdimensions of interpersonal anger responses. However, irritable temperament, which is negatively associated with the offensive subdimension of anger-related behaviors, does not reflect the expected result; the low sample size may have caused this result. We stated a positive correlation between the revenge subdimension for interpersonal anger reactions and anxious temperament. However, although it was expected that the results of the research would be significant in the anxious subdimension, this result was not obtained; this result possibly changes as the number of samples and the number of patients with an anxious temperament increase.

Limitations

The first limitation of our study was the small sample size, as mentioned above. Furthermore, the heterogeneity level was not sufficient because the study was conducted only in Ordu City. Therefore, the results may have affected the generalization of the population because of both the insufficient number of samples and the homogeneity of the sample. Another limitation was that multiple scales were simultaneously used. According to the observations made, patients may have been bored and had difficulty filling in the scales. As these were self-report scales, the answers received were evaluated on the assumption of sincerity.

CONCLUSION

This study elucidates anger dimensions such as anger-related thoughts, behaviors, and reactions in individuals who received retinol treatment for AV. In addition to anger and its dimensions, temperament was also investigated. Despite all limitations, this study attempted not only to obtain general data about anger but also to elucidate anger dimensions such as anger-related thoughts, behaviors, and reactions in individuals who received retinol treatment used in AV disease. Although several studies have investigated the relationship between AV and psychiatric symptoms, no research has been reported regarding the relationship between anger dimensions and temperament after retinol treatment which might give our study an original and valuable contribution to the literature. Well diagnosed, well cared for. Considering that there are few studies on this subject in the literature, it is obvious that more studies on the psychiatric effects of isotretinoin, which is highly effective in the treatment of severe and persistent AV, are needed.

ETHICAL APPROVAL

This study was approved by the Ordu University, Ordu, Turkey (approval no: #2014/01).

AUTHORS' CONTRIBUTIONS

EYD: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ÖKK:** Data curation, Visualization.

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ORIGINAL ARTICLE

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Human papillomavirus DNA testing with the urine sample is not yet available: the accuracy of two distinct kits

Ferah Kazanci[1*], Ozlem Erdem[2], Mehmet Anil Onan[1], Gulendam Bozdayi[3]

SUMMARY

OBJECTIVE: The aim of this study was to assess the results and efficiency of two real-time polymerase chain reaction procedures for detecting human papillomavirus utilizing urine samples.

METHODS: This study comprised 151 patients who had previously tested positive for human papillomavirus in their cervical samples. Two different commercial real-time polymerase chain reaction techniques were used for identification and genotyping human papillomavirus in urine specimens. The urine samples of 151 patients were evaluated via the Roche Cobas test, and the urine samples of 91 patients were also evaluated via the Qiagen test.

RESULTS: The overall consistency of urine and cervical swab specimens for the identification of human papillomavirus in Roche Cobas and Qiagen tests were 44.8 and 44%, respectively. The rates of positive human papillomavirus results from urine samples were 57 and 70.3%, respectively. The overall concordance among Roche Cobas and Qiagen tests utilizing urine samples for human papillomavirus type 16/18 was 84.3% with a kappa value of 0.675, and for other high-risk-human papillomavirus, it was 75.60% with a kappa value of 0.535. Roche Cobas showed high concordance with Qiagen test.

CONCLUSION: human papillomavirus positivity was not detected in all urine samples. It is still inappropriate to recommend the use of urine liquid biopsy for the accurate and reliable detection of human papillomavirus. Due to the lack of a standardized tool, the utilization of urine samples as a screening human papillomavirus test remains a challenge.

KEYWORDS: Pap smear. Human papillomavirus. Real time PCR. Urine collections.

INTRODUCTION

Screening programs should have been standardized, practical, sufficient, effective, and acceptable for the target population. Cervical neoplasm is the fourth common cancer, causing deaths in females of poor- and moderate-income countries[1]. Scientific and demographic studies have obviously shown that persistent HPV infection is a risk factor for the occurrence of both pre-invasive cervical disorders and invasive carcinoma[2]. HPV detection for cervical cancer screening has different benefits compared with that for cytology-based screening, which comprises heightened sensitivity and improved diagnostic repeatability in many environments, but it is an invasive method[2]. The utilization of urine liquid biopsy as self-collection of specimens for HPV detection has been demonstrated to be very suitable in various cultures due to many reasons, such as the ability to apply samples outside the health center, being a noninvasive method, and the facility to access and increase the screening uptake[3-5]. However, due to the lack of a standardized tool, the utilization of urine samples for screening HPV test remains a challenge.

The purpose of this trial was to determine the efficiency of HPV determination in urine samples of the patients who had prior HPV-positive results in the cervical swab specimens.

METHODS

Study design and characterization of participants

The Department of Gynecologic Oncology at the Gazi University Faculty of Medicine Hospital carried out this prospective investigation. A total of 151 patients with previous HPV DNA-positive results in the cervical swab specimens that were taken by clinicians between January 2019 and January 2021 were considered in this trial. Besides, the demographic data such as age, body mass index (BMI), the number of pregnancy and parity, the route of parturition, first coitus age, the history of smoking and using of oral contraceptives (OC), and the pathological findings in cervical cytology were also evaluated. None of them had undergone hysterectomy or received prior

[1]Gazi University, Faculty of Medicine, Department of Gynecologic Oncology – Ankara, Turkey.
[2]Gazi University, Faculty of Medicine, Department of Pathology – Ankara, Turkey.
[3]Gazi University, Faculty of Medicine, Department of Medical Microbiology, Division of Medical Virology – Ankara, Turkey.
*Corresponding author: ferahkazanci@hotmail.com

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treatment for cervical disorders or cancer. They had no history of HIV or other sexually transmitted infections and were not pregnant at the time of trial.

Detection of human papillomavirus DNA in cervical swab and urine specimens

Cervical swab specimens

Cervical specimens were gathered by an gynecologist oncologist via a cervical swab, stored in a PCR Cell Cobas medium vial (produced by Roche Diagnostics, USA), and sent to the laboratory. Cervical swabs are steady at 2–8°C for testing with the Cobas 4800 HPV test kit (Roche Cobas 4800 HPV Test Package Insert 2010). Real-time PCR technology is integrated with completely automated specimen preparation in the Cobas 4800 platform. The test is created to isolate, replicate, and identify a wide range of high-risk HPV (HR-HPV) genotypes. The test can identify 14 HR-HPV genotypes in a single assay by presenting individual scores for high-risk genotypes: HPV 16 and HPV 18 and other HR-HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)[6].

Urine samples

The first stream urine samples of the HPV-positive patients were collected in a sterile cup and sent to the laboratory. A minimum of 20 mL of every urine specimen was separated by centrifugation at 4000×g for 10 min. After vortexing, the concrete pellet was redissolved in a volume of 5 mL of supernatant and relocated to one phial of PCR collection medium. After this procedure, urine specimens were analyzed with real-time PCR techniques. Two different commercial kits (Roche Cobas and Qiagen) were employed for the determination of HPV DNA in urine specimens. Qiagen could detect 14 HR-HPV genotypes (16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59, 66, 67, and 68)[6,7]. DNA extraction from urine samples was carried out in an automatic device (EZ1, Qiagen, Germany) using the EZ1 Virus Mini Kit. Extracted DNA samples were studied with two different commercial real-time PCR kits on a real-time PCR device (Rotor-Gene Q, Qiagen, Germany). Four different florescent dyes (green), Joe (yellow), Rox (orange), and Cy5 (red) channels were used for the determination of HPV genotypes.

Statistical analysis

SPSS version 21.0 was used to conduct the statistical analysis (SPSS Inc., Chicago, IL, USA). Descriptive statistical analysis was quantified for the overall population, in addition to both the urinary HPV-positive and -negative groups. The sensitivity of HPV determination in urine specimens, checked for the identification in cervical samples, was computed as percentages. Chi-square test for competition pairs (McNemar test) was used to crosscheck the efficiency of the two types of samples concerning the determination of HPV types. The kappa correlation coefficient was computed for the correlation analysis. Outcomes were considered statistically significant at a $p<0.05$.

Ethical approval and informed consent

The Ethics Committee of the Faculty of Medicine of Gazi University consented to the research (date: 16.10.2018, decision number: 795). Written informed permission was obtained after all participants received information about the study. This research was carried out in accordance with the Helsinki Declaration principles.

RESULTS

In this research, the median age of the cases was 40 years (ranged from 23 to 60), and most of them were between 30 and 50 years old (71.5%). The demographic features of patients are listed in Table 1. There were 151 and 91 urine samples examined with the Roche Cobas and Qiagen tests, but 17 and 7 of the study population were excluded from each test due to an invalid result, respectively. Therefore, 134 and 84 urine samples

Table 1. Distribution of human papillomavirus genotypes, histopathological results, and demographic data of human papillomavirus-positive patients in cervical samples.

	n, %
Age (median/min–max) (years)	40 (8/23–66)
Nulliparous/ birth child	30 (19.9)/121 (80.1)
Route of parturition (VD/C-S)	75 (49.7)/46 (30.5)
BMI (average)	29.23 (21–53)
First coitus age (min–max) (years)	20 (14–38)
Smoking status (none/active smoking)	81 (53.6)/70 (46.3)
Using oral contraceptive status (−)/(+)	120 (79.5)/31 (20.5)
HPV type	
16/18 OHR	44 (29.1)/15 (9.9)/58 (38.4)
16+OHR/18+OHR/16+18+OHR	27 (17.9)/6 (4)/1 (0.7)
Cervical cytology	
Malignancy(−)/ASCUS/ASC-H	80 (53%)/30 (19.9%)/3 (2%)
LSIL/HSIL	33 (21.9%)/5 (3.3%)
Total	151

VD: vaginal delivery; C-S: cesarean section; ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesions; HSIL: high-grade squamous intraepithelial lesions; ASC-H: atypical squamous cells (cannot exclude HSIL).

were studied by Roche Cobas and Qiagen tests, respectively, and,then compared.

The frequencies of HPV DNA in urine samples that were studied using Roche Cobas and Qiagen kits were 57 and 70.3%, respectively. The sensitivities of Roche Cobas and Qiagen tests were 64.2 and 76.2%, respectively. The overall consistency between urine and cervical swab specimens for the identification of HPV in Roche Cobas and Qiagen tests was 44.8% and 44%, with a kappa value of 0.321 and 0.314, respectively. The overall accuracy to detect HPV DNA, between Roche Cobas and Qiagen tests, was 77.3%, with a kappa value of 0.504 (p<0.001).

The highest prevalent HPV subtype was OHR, with 38.4% rate in cervical samples (Table 1). Furthermore, in urine samples, the highest rates belong to OHR-HPV types in both Roche Cobas and Qiagen tests (27.2 and 16.6%) (Figure 1).

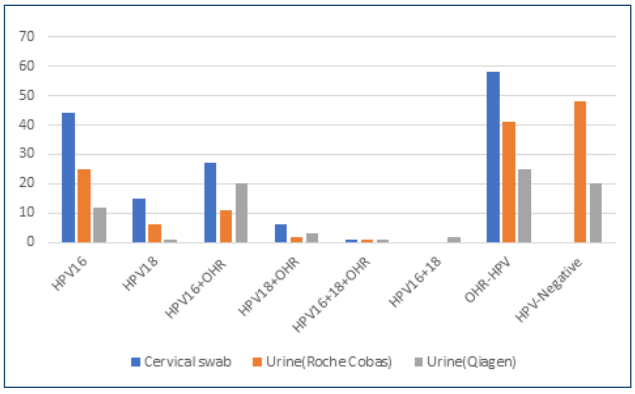

Figure 1. Distribution of human papillomavirus genotypes in cervical swab and urine samples with the result of two different tests.

The accuracy among the Roche Cobas and Qiagen tests utilizing urine samples for HPV type 16/18 was 84.3% with a kappa value of 0.675, and that for OHR-HPV type was 75.60% with a kappa value of 0.535 (Table 2). There was no statistical significance in urine samples of HPV-positive and HPV-negative patients between analysis via Roche Cobas and Qiagen tests in terms of demographic features such as age (p=0.354 and p=0.914), parity status (p=0.565 and p=0.976), BMI (p=0.850 and p=0.967), smoking behaviors (p=0.542 and p=0.075), using oral contraceptives (p=0.159 and p=0.376), and first coitus age (p=0.656 and p=0.319).

DISCUSSION

The most essential strategy for eradicating cervical cancer is to screen women by an HPV test with cervical cytology samples[3]. Unfortunately, many women ignored these programs or continued many years without being screened because of different reasons, such as difficulty to access medical centers in developing countries, busy work schedule, time constraints, and socio-economic anxiety in developed countries. Furthermore, obstacles to testing include absence of information, personal choice, anxiety, shame, and honesty in the health care system. Besides, the detection of HPV with cervical screening has brought some limitations for single, sexually inactive women and adolescents who do not wish to have a vaginal examination[5,8]. Due to all these unfavorable reasons, new strategies are

Table 2. Accuracy in human papillomavirus DNA detection results between Roche Cobas and Qiagen tests using urine samples.

HPV genotypes		Qiagen HPV(+)	QiagenHPV(−)	Diagnostic accuracy	Kappa value	95%CI kappa value	p-value
16 and/or 18	Roche HPV(+)	27	1	84.30%	0.675	0.409–0.941	p<0.001
	Roche HPV(−)	7	16				
OHR	Roche HPV(+)	12	0	75.60%	0.535	0.250–0.820	p<0.001
	Roche HPV(−)	9	16				
		16 and/or 18(+)	OHR(+)				
16 and/or 18	Roche HPV(+)	27	0	92.80%	0.837	0.538–1.136	*p<0.001
OHR	Roche HPV(−)	3	12				

*McNemar test, p<0.05.

needed to facilitate participation in cervical cancer screening. The utilization of urine as liquid biopsy for the detection of HPV has been demonstrated to be extremely favorable because of similarly strong association between urinary and cervical HPV DNA, easy collection of samples, and relatively high suitability for women[9-11].

HPV DNA testing in urine samples presents some obstacles because of so many variables, such as urine collection method, storage situations, centrifugation process, and DNA isolation or amplification procedure[10]. Currently, there are various HPV genotyping methods for identifying DNA, such as PCR, real-time PCR, restriction fragment length polymorphism (RFLP), hybrid capture, and linear array[9]. In this investigation, we compared the abilities of the Cobas 4800 HPV test and the Qiagen test to identify HPV DNA in urine samples. These two real-time PCR assays have a variety of benefits over existing HPV genotyping and/or detection bioassays. The outcomes can be acquired nearly 4–6 weeks after receiving the cervical samples in these assays. The overall agreement between urine and cervical swab samples for the detection of HPV in Roche Cobas and Qiagen tests was 44.8% and 44%, with a kappa of 0.321 and 0.314, respectively, in this study. Bernal et al., determined 88% agreement between urine and cervical samples. Bernal et al., applied the PCR method with Roche Cobas 4800 HPV test on matched cervical and first voided urine in 125 patients between the ages of 21 and 65 years[12]. In addition, Hagihara et al., reported that the concurrence among the urine and cervical samples was 98.4%, with a kappa of 0.792. Hagihara et al., detected DNA by the PCR method with the Anyplex™ HPV28 kit on synchronous cervical and urine samples in 240 patients between the ages of 19 and 58 years[9]. High correlation was observed in both studies with simultaneous HPV DNA detection in cervical and urine samples[9]. In this study, urine samples were collected from HPV-positive patients in cervical swab when the patients came to get information about their cervical cytology results. The reason of low sensitivity to detect HPV in urine samples is that the time interval between the collection time of urine samples and cervical cytology was longer. During this period, the patients may be in the recovering period. The overall concordance among the Roche Cobas and Qiagen tests utilizing urine samples for HPV type 16/18 was 84.3% with a kappa value of 0.675, and that for OHR-HPV was 75.60% with a kappa value of 0.535. Roche Cobas showed high concordance with Qiagen test. However, three samples that were detected as type 16 and/or 18 by the Qiagen test were detected as OHR-HPV by the Roche Cobas test (Table 2). Lim et al., demonstrated a similar agreement to detect HPV 16/18 between Roche Cobas and Abbott (relative sensitivities: 79.2% and 81.8%) in their study[13]. In this and Lim's studies, we determined that different kits did not influence the HPV detection rate in urine samples determined by the PCR technique.

There are also some confusions in the description of first void urine, frequently thought to be the first urine of the day[11]. In our study, first void urine was defined as the collection of the initial urine (not midstream urine) at any time of the day. Recent studies demonstrated significantly higher level of HPV DNA in the first part of void urine than in the subsequent part[14]. Furthermore, recent studies determined that there was no significant impact on time of collection between morning and later during the day[15,16]. However, we had no information about the interval time of two urinations in terms of viral DNA accumulation in our cases, which is the study's limitation. When the interval between two urinations is long, the rate of detecting excess HPV DNA may increase due to the increased accumulation of infected cells in the cervical discharge.

In addition, the collection device affects the ratio of the HPV detection level. Pattyn J. demonstrated that the HPV concentrations were observed higher in the Colli-Pee® device than the standard urine cup. During transport, storage, and pre-analytical processing steps, the collection of a nucleic acid preservative for urine samples provides effective and accurate results as it prevents degradation of cell-associated and cell-free DNA by nucleases[15]. Another reason for low sensitivity in our study is that we collected the urine samples in a sterile urine cup without any preservative medium.

In this study, no statistical significant differences were observed in terms of age, parity, types of parturition, body mass index, smoking status, first coitus age, or contraceptive method when comparing patients with a positive HPV to those with a negative result in urine samples which were analyzed by Roche Cobas and Qiagen tests similar to the study by Nicolau et al.[17].

CONCLUSION

The detection rate of HPV in urine specimens is lower than that in cervical samples. Many factors such as the urine collection methods, storage situations, centrifugation process, DNA extraction, or amplification techniques may be responsible for the low DNA level in urine samples. Beside this, Roche Cobas and Qiagen tests showed high concordant results, including genotyping. An approach for testing HPV using urine samples is not yet available.

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AUTHORS' CONTRIBUTIONS

MAO: Conceptualization, Methodology, Writing – review & editing. **FK:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **OE:** Data curation, Writing – review & editing. **GB:** Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Use of high-dose steroid therapy: addition of anakinra in the treatment of severe COVID-19

Kadir Gorkem Guclu[1*], Ceyda Geyiktepe-Guclu[2], Osman Faruk Bayramlar[3], Gulsah Tuncer[4], Mehtap Aydin[1]

SUMMARY

OBJECTIVE: The aim of this study was to compare the clinical effects of the addition of anakinra to high-dose steroid therapy in COVID-19 patients with macrophage activation syndrome.

METHODS: This was a single-center retrospective study conducted in Ümraniye Training and Research Hospital between March 11, 2020, and April 28, 2021. Patients receiving only high-dose steroid or anakinra+steroid were enrolled. The first day of anakinra was considered as day 0. Laboratory values and oxygen requirements were followed up for 7 days. Patients were divided into two groups: 66 patients in the high-dose steroid group and 67 patients in the anakinra+steroid group. The primary outcome was 28-day mortality.

RESULTS: After treatment, a significant decrease in ferritin levels was detected only in the anakinra+steroid group (p=0.001). In both groups, there were significant changes in lymphocytes, C-reactive protein, lactate dehydrogenase, and fibrinogen levels during the 7-day follow-up. Changes in oxygen status according to the World Health Organization clinical scale on day 3 and day 7 between high-dose steroid and anakinra+steroid groups were similar (p=0.976). Complications were higher in the anakinra+steroid group than in the steroid group (26% vs. 12%, p=0.03). The rates of 28-day mortality were 57% in the anakinra+steroid group and 42% in the high-dose steroid group (p=0.48). In multivariate regression, anakinra did not affect 28-day mortality (p=0.67).

CONCLUSION: The addition of anakinra to steroid treatment resulted in a significant decrease in biochemical parameters. However, no significant difference was observed in the oxygen status between the groups. The addition of anakinra to steroid treatment did not decrease mortality. Clinicians should be aware of the complications of anti-inflammatory therapies.

KEYWORDS: COVID-19. Anakinra. Steroid. Cytokine storm.

INTRODUCTION

The global spread of COVID-19 has affected nearly every country, resulting in a significant number of fatalities, with approximately 6.95 million deaths reported worldwide[1]. Certain laboratory parameters, such as C-reactive protein (CRP), lymphopenia, increased ferritin levels, and thrombocytopenia, have been identified as indicators of poor prognosis in COVID-19 cases[2]. The progression of COVID-19 is often accompanied by an upsurge in proinflammatory endogenous cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha). This situation can lead to MAS, both of which have been linked to severe morbidity and mortality[3]. Notably, studies suggest that anakinra, an IL-1 receptor antagonist, may serve as a potential treatment option for cytokine storms and MAS in COVID-19[4]. This study was conducted to evaluate the clinical outcomes of adding anakinra to high-dose steroid therapy in patients diagnosed with MAS based on clinical and laboratory findings. Our study aims to compare the use of anakinra with high-dose steroid to the use of only high-dose steroid, with an objective of determining any clinical benefits.

METHODS

Study design

This was a single-center retrospective study. We included patients who were admitted to the Hospital between March 11, 2020 and April 28, 2021 for COVID-19 management and follow-up. MAS treatment was given in accordance with

[1]Umraniye Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology – İstanbul, Turkey.
[2]Haseki Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology – İstanbul, Turkey.
[3]Turkish Ministry of Health - Istanbul Health Directorate, Bakırköy District Health Directorate – İstanbul, Turkey.
[4]Bilecik Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology – Bilecik, Turkey.
*Corresponding author: gorkemguclurd@gmail.com
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the COVID-19 guidelines of the Ministry of Health. These recommendations include only high-dose steroid or anakinra with high-dose steroid. According to the guidelines, anakinra was administered intravenously at a maximum dose of 1200 mg/day. The total dose was divided into two or three doses and administered intravenously at equal time intervals. Patients with SpO_2 <93% or PaO_2/FiO_2 <300, CRP >50 mg/L, and ferritin >600 ng/mL or IL-6 >6 ng/µg were considered for MAS treatment. On the day of MAS evaluation, both patient groups were given high-dose steroid. All patients were given 250 mg of methylprednisolone for 3 days. Subsequent doses were reduced according to the clinical situation. Some patients had anakinra with 250 mg of methylprednisolone[5]. Anakinra was added to the treatment of patients who did not have clinical improvement, according to the Ministry of Health guidelines. After the development of MAS, laboratory values and oxygen status for 7 days were retrospectively collected. In addition, both groups were underlying chronic diseases, and clinical and laboratory findings during admission and CT screening results were retrospectively collected. According to the COVID-19 guidelines, at least 50% involvement in unilateral or bilateral was determined as severe CT findings.

These patients were divided into two groups: the first group comprised patients receiving high-dose steroid (steroid group), while the second group comprised patients receiving anakinra in addition to high-dose steroid (anakinra+steroid group). The day of addition of anakinra was considered as day 0 in patients who were added to the anakinra group. Laboratory values and oxygen requirements were compared for 7 days (days 0, 1, 3, 5, and 7). A total of 66 patients were included in the steroid group, and 67 patients were included in the anakinra+steroid group. The primary outcome was 28-day mortality. The secondary outcomes were changes in laboratory values and the WHO's COVID-19 clinical scale. The 28-day mortality rates and intensive follow-up were examined. Patients were grouped according to their oxygen status, as suggested by the WHO's COVID-19 clinical scale[6].

Study population

In our study, patients with laboratory-confirmed COVID-19 who developed MAS with clinical and laboratory findings were included. The inclusion criteria for this study were as follows: older than 18 years, patients with developing MAS, and laboratory-confirmed COVID-19. Laboratory-confirmed COVID-19 was defined as patients with a positive SARS-CoV-2 test at least once in oropharyngeal and nasopharyngeal swab samples. The exclusion criteria for this study were as follows: HIV -positive patients, patients with COVID-19 vaccines, and patients with viral hepatitis, active tuberculosis, acute respiratory distress syndrome (ARDS), and neutropenia and uncontrolled diabetes. In addition, patients who died within the first 3 days after steroid or anakinra administration or patients in need of intensive care during admission were excluded. Pregnant patients and patients ≥6 points on the clinical scale of the WHO were excluded[6].

Statistical analysis

The SPSS 20 Windows package program (IBM Corp., Armonk, NY, USA) was utilized for data analysis. Key variables included 28-day mortality, the impact of anakinra supplementation on oxygen demand, and laboratory values. Various factors such as sex, age, comorbidities, laboratory values, CT findings, and oxygen status were compared. Categorical variables were presented as numbers and percentages, while continuous variables were expressed as mean±SD or median with interquartile range (IQR). The chi-square test was used to compare categorical variables, while Student's t-test or Mann-Whitney U test was employed for numerical variables depending on their distribution. Statistical significance was set at $p<0.05$. Variables that exhibited a significant effect on 28-day mortality were included in the univariate logistic regression analysis. In the multivariate analysis, parameters that were statistically significant in the univariate analysis and had an odds ratio of ≥1.01 were considered. The obtained results were assessed with a 95% confidence interval.

RESULTS

Of the included patients, 62.4% were males and 37.6% were females, with a mean age of 59±14 years. The patient population was divided into two groups: 67 patients in the anakinra+steroid group and 66 patients in the steroid group. The distribution of age, gender, and chronic disease was similar between the two groups (p=0.43) (Table 1). None of the patients had received any COVID-19 vaccine. Severe CT findings were observed in 62.3% of patients in the anakinra+steroid group and 37.7% in the high-dose steroid group (p=0.03). At admission, oxygen requirement, duration of symptoms, fever, pulse rate, and respiratory rate were evaluated, and no significant differences were observed between the two groups (Table 1). At admission, laboratory parameters such as leukocyte count, lymphocyte count, platelet count, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), C-reactive protein (CRP), D-dimer, and fibrinogen levels were similar in both groups.

Moreover, mean ferritin levels were higher in patients receiving anakinra+steroid compared to those receiving only

Table 1. Demographic data of patients, comorbidities, tomographic findings, and vital signs.

Characteristics	Steroid (n=66)	Anakinra+steroid (n=67)	p-value
Age (mean±SD)	60±14	57±14	0.38
Sex			
-Male	39 (47)	44 (53)	0.43
-Female	27 (54)	23 (46)	
Comorbidities	48 (53)	41 (46)	
-Chronic lung disease	10 (59)	7 (41)	0.44
-Diabetes	20 (45)	24 (55)	0.50
-Hypertension	30 (53)	27 (47)	0.55
-Cardiovascular diseases	9 (47)	10 (53)	0.83
-Heart failure	3 (42)	4 (57)	1.00
-Central nervous system diseases	2 (50)	2 (50)	1.00
-Cancer	3 (37.5)	5 (62.5)	0.72
-Chronic kidney disease	4 (57)	3 (43)	0.72
-Rheumatic diseases	0 (0)	2 (100)	0.50
Tomographic findings			
-Mild	46	34 (42.5)	**0.03**
-Severe	(57.5) 20 (38)	33 (62)	
Fever (°C) (mean±SD)	36.9±0.7	36.8±0.6	0.30
Respiratory rate/minute (mean±SD)	24±4	23±4	0.28
Pulse/minute (mean±SD)	88±15.4	90±15.2	0.66
Complaint period (day) (mean±SD)	6±3	7±4	0.58
Admission saturation (%) (mean±SD)	88±5	88±6	0.10

Bold indicates statistically significant p-value.

steroid at admission (1136±1323 ng/mL vs. 634±574 ng/mL, p=0.03). ALT levels were higher at admission in the anakinra group (47±46 IU/L vs. 31±21 IU/L, p=0.03). When anti-inflammatory therapy started, leukocyte, thrombocyte, ALT, LDH, and ferritin levels were higher in the anakinra+steroid group. CRP levels were higher in the steroid group (p=0.19). There was no difference in fibrinogen, IL-6, D-dimer, CK, and AST values. The mean IL-6 levels were found to be 41.8±36 pg/mL in the anakinra+steroid group and 27.8±27.5 pg/mL in the steroid group (p=0.21). Intensive care admission was higher in the anakinra+steroid group (68% vs. 32%, p=0.09).

Anakinra was started on the day 11 of symptom onset, and steroids were started on day 9. The mean procalcitonin values measured on the day of treatment were <0.25 ng/mL, and no significant difference was found between the two groups. In both groups, there was no significant change in leukocyte, ALT, CK, or D-dimer levels for 7 days. The decrease in ferritin levels was significant only in the anakinra+steroid group (p=0.001). LDH decrease was observed in both groups (Table 2). The LDH decrease was more significant in the anakinra+steroid group (p=0.001 vs. p=0.005).

Oxygen status on days 3 and 7 after anti-inflammatory therapy was classified and compared according to the WHO clinical scale. Changes in the oxygen status according to the WHO clinical scale on days 3 and 7 between the high-dose steroid and anakinra+steroid groups were similar (p=0.976). Complications developed in 26 of 133 patients. Bacterial pneumonia was observed in 10 patients, bacteremia in 4 patients, ARDS in 9 patients, gastrointestinal system bleeding in two patients, and psychiatric problems in 3 patients. Complication rates were 26% in the anakinra+steroid group and 12% in the steroid group (p=0.03).

In the 28-day follow-up of the patients, 57% of the patients who died were detected in the anakinra+steroid group and 42% were in the steroid group, and no significant difference was observed between the 28-day mortality rates (p=0.48).

Table 2. Laboratory findings on days 1, 3, 5, and 7.

Parameters		Anakinra+steroid		p	Steroid		p
		Mean±SD	Median (IQR)		Mean±SD	Median (IQR)	
Platelet count/mm^3	Day 1	329±142	316 (236–384)	**0.03**	267±92	270 (198–306)	**0.01**
	Day 3	349±126	330 (264–401)		306±119	306 (235–375)	
	Day 5	358±131	351 (278–411)		336±137	327 (267–402)	
	Day 7	341±114	329 (274–395)		316±132	309 (216–370)	
Lymphocyte count/mm^3	Day 1	774±489	670 (500–890)	**0.001**	792±420	710 (500–1000)	**0.001**
	Day 3	947±669	755 (540–1200)		953±575	815 (525–1325)	
	Day 5	1052±785	920 (530–1280)		1112±706	990 (450–1530)	
	Day 7	1300±1011	1010 (630–1610)		1144±860	1100 (435–1410)	
AST, IU/L	Day 1	40±28	32 (23–43)	**0.03**	43±60	28 (21–49)	**0.08**
	Day 3	39±21	34 (22–52)		44±52	32 (21–44)	
	Day 5	37±21	34 (22–45)		32±17	27 (20–39)	
	Day 7	31±19	24 (19–33)		30±18	23 (19–35)	
	Day 3	69±46	61 (31–98)		58±63	40 (24–76)	
	Day 5	80±58	63 (34–106)		57±47	45 (26–80)	
	Day 7	74±61	49 (29–114)		74±84	51 (29–86)	
LDH, IU/L	Day 1	458±214	418 (310–568)	**0.001**	339±100	326 (286–390)	**0.005**
	Day 3	461±220	401 (333–558)		363±119	339 (276–421)	
	Day 5	400±186	357 (281–485)		313±132	293 (243–349)	
	Day 7	382±234	329 (259–429)		323±118	320 (264–358)	
Ferritin, ng/mL	Day 1	1361±1532	766 (432–2100)	**0.001**	746±665	572 (267–982)	0.40
	Day 3	1064±1000	781 (398–1411)		751±763	548 (310–928)	
	Day 5	925±837	700 (304–1213)		682±654	500 (312–814)	
	Day 7	761±614	583 (328–992)		697±564	478 (360–983)	
CRP, mg/L	Day 1	48±37	38 (19–66)	**0.001**	55±39	52 (28–75)	**0.001**
	Day 3	34±36	20 (10–45)		39±56	23 (12–42)	
	Day 5	32±37	12 (6–53)		32±69	15 (8–26)	
	Day 7	35±61	8 (3–30)		19.8±23.4	10.5 (4–27)	
Fibrinogen, mg/dL	Day 1	599±118	607 (504–675)	**0.001**	532±127	515 (464–610)	**0.001**
	Day 3	522±133	520 (415–607)		507±139	496 (405–563)	
	Day 5	493±128	475 (411–580)		496±137	470 (437–532)	
	Day 7	463±144	441 (376–523)		438±101	428 (361–529)	

IQR: interquartile range; AST: aspartate transaminase; LDH: lactate dehydrogenase; CRP: C-reactive protein. Bold indicates statistically significant p-value.

Univariate regression analysis for affecting 28-day mortality, including age, sex, severity of CT findings, LDH, ALT, CRP, and ferritin levels, duration of steroid administration, and addition of anakinra, was performed (Table 3). Age, severe CT findings, and duration of steroid administration were found to be the factors affecting 28-day mortality. Multivariate regression analysis of anakinra addition was performed with factors affecting mortality, such as age, severity of CT findings, and LDH level. As a result of the regression analysis performed with these confounding factors, it was found that the addition of anakinra did not affect 28-day mortality (p=0.67).

DISCUSSION

In our study, the addition of anakinra to steroid treatment resulted in a significant decrease in the ferritin level in the

Table 3. Univariate and multivariate analysis of parameters predicted to be effective on mortality.

Parameters	Univariate analysis			Multivariate analysis		
	OR	CI	p	OR	CI	p
Receiving anakinra	0.714	0.263–1.876	0.48	0.765	0.223–2.631	0.67
Age	1.052	1010–1.086	**0.02**	1.044	1.010–1.086	**0.03**
Ferritin, ng/mL	1.000	0.999–1.000	0.57			
ALT, IU/L	0.995	0.645–1.010	0.50			
CRP, mg/L	1.000	0.645–1.010	0.50			
LDH, IU/L	1.010	1.000–1.010	0.09			
Duration of steroid	1.123	1.103–1.237	**0.02**			
Severity of CT finding	1.754	0.344–2.941	**0.03**	3.100	1.058–9.300	**0.03**
Sex	0.970	0.357–2.631	0.94			

OR: odds ratio; CI: confidence interval; ALT: alanine transaminase; CRP: C-reactive protein; LDH: lactate dehydrogenase; CT: computed tomography. Bold indicates statistically significant p-value.

first 7 days in patients. The ferritin and LDH values of the patients, which were checked at the time of admission to the hospital and on the day of MAS evaluation, were higher in the anakinra+steroid group, and there may be a bias against anakinra. Nevertheless, laboratory values were followed up for 7 days after anti-inflammatory treatment, and a significant decrease in ferritin levels was detected in the anakinra+steroid group (p=0.001). The effect of anakinra on ferritin decrease is consistent with the literature[5,6]. In a prospective cohort study of 60 critically ill COVID-19 patients followed up in the intensive care unit, ferritin reduction was more pronounced in the anakinra group. In the study by Emma et al., it was shown that the hyperinflammatory state regressed more rapidly in patients who were given anakinra[7]. Anakinra more effectively decreases the ferritin levels by inhibiting cytokine release and the inflammatory cycle with IL-1 blockade[4]. LDH reduction was found to be significant in both groups. In the literature, it is emphasized that high LDH levels may be associated with more severe disease course, hospitalization, and the need for intensive care[8]. LDH decrease was observed in the anakinra+steroid (p=0.001) and steroid (p=0.005) groups.

In our study, anakinra was administered intravenously, with a mortality rate of 16%. In the study by Huet et al., 100 mg of anakinra was administered subcutaneously twice a day in patients with saturation <93% and who did not need intensive care, and the mortality rate was found to be 25% at the 20-day follow-up. Also, steroid was not given to every patient in the control group[9]. In our study, mortality in the anakinra+steroid group was lower than that in other studies. This may be due to intravenous administration of anakinra. With the intravenous use of anakinra, there was no absorption problem, and the effective dose was reached quickly[10]. In our study, the mortality rate in the steroid group was 12%. In the study by Batirel et al., conducted with 189 patients, 250 mg methylprednisolone treatment for 3 days was compared with 6 mg dexamethasone treatment. In their study, while mortality was 5% in the pulse steroid arm, it was 11% in the 6 mg dexamethasone arm[11]. The reason for low mortality in their study was that it was accepted in mild patients and nondeveloping MAS. In our study, adding anakinra to treatment did not affect 28-day mortality (p=0.67). In a systematic review in which the efficacy of anakinra was evaluated in hospitalized patients with COVID-19, mortality did not decrease compared to placebo and standard treatment groups in patients receiving anakinra[12]. In another study, clinical outcomes were not better in patients receiving anakinra than the standard care group and placebo group[13].

Our study had some limitations. First, it was conducted in a single center, which may limit the generalizability of the findings. Second, the anakinra+steroid group had the worst oxygen status before treatment. This could be attributed to the fact that anakinra was administered to patients with higher ferritin levels and poorer clinical conditions, potentially introducing selection bias. Additionally, further studies are required to investigate the effects of anakinra in more severe patient groups who require mechanical ventilation.

However, this study had several strengths. First, regular follow-ups of routine tests, IL-6, CK, and procalcitonin were performed regularly. Oxygen status was observed regularly for 7 days. However, data were collected retrospectively. Second, only the critical patient group, before going to the intensive care unit, was discussed.

CONCLUSION

COVID-19 progresses to MAS with proinflammatory cytokine increase. In our study, the addition of anakinra to steroid treatment resulted in a significant decrease in ferritin and LDH levels in the first 7 days in patients who developed cytokine storm. However, multivariate analysis of the addition of anakinra found no effect on 28-day mortality. There was no significant difference in the magnitude of improvement in the oxygen status between the two treatment groups, according to the WHO's clinical scale. Clinicians should be aware of the complications of anti-inflammatory therapies. However, more controlled studies are needed to determine the effectiveness of high-dose steroid administration with anakinra.

AUTHORS' CONTRIBUTIONS

KGG: Conceptualization, Data curation, Methodology, Validation. **CGG:** Conceptualization, Data curation, Methodology. **OFB:** Formal Analysis, Software. **GT:** Writing – review & editing. **MA:** Writing – review & editing.

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ORIGINAL ARTICLE

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Study on symptom dimensions and clinical characteristics in patients with obsessive-compulsive disorder

Xuan Liu[1,2,3], Yuehan Zhao[3], Pengchong Wang[1,2], Xiangyun Yang[1,2], Zhanjiang Li[1,2*]

SUMMARY

BACKGROUND AND OBJECTIVE: The aim of this study was to explore the symptom dimensions and clinical characteristics of obsessive-compulsive disorder in the context of Chinese culture.

METHODS: In this cross-sectional study, the severity of obsessive-compulsive symptoms, the distribution of symptoms, and symptom scores of 263 patients with obsessive-compulsive disorder were assessed using the Yale-Brown Obsessive-Compulsive Scale and Yale-Brown Obsessive-Compulsive Inventory Symptoms Checklist. System cluster analysis and Pearson analysis were performed to explore the relationships between the main clinical characteristics and symptom dimensions.

RESULTS: Cluster analysis identified four symptom dimensions of obsessive-compulsive disorder: (1) symmetry precision; (2) contamination cleaning; (3) aggression examination; and (4) taboo thinking. The symmetry precision dimension showed an association with years of education. The compulsive score, total Yale-Brown Obsessive Compulsive Scale score, contamination cleaning dimension, and aggression examination dimension had significant relationships. Age, age at onset, obsessive score, and compulsive score had a significant correlation with the taboo-thinking dimension.

CONCLUSION: The symptom dimensions of obsessive-compulsive disorder in China are similar to those in other regions. Each of the four symptom dimensions had distinct clinical characteristics.

KEYWORDS: Obsessive-compulsive disorder (OCD). Symptom. Cross-sectional studies. Cluster analysis.

INTRODUCTION

Obsessive-compulsive disorder (OCD) is a common psychiatric disorder characterized by obsessions (recurrent intrusive thoughts with excessive anxiety) and compulsions (excessive repetitive actions used to reduce obsession-induced anxiety)[1,2]. Approximately 3% of the world's population is affected by OCD[3], resulting in high social and economic burden[4]. Increasing evidence has suggested that OCD is an extremely heterogeneous mental disorder[5]. Patients with the same definite diagnosis of OCD may have very different clinical manifestations[6], which may be related to different genetic and neurobiological mechanisms, resulting in different onset characteristics, manifestations, treatment methods, effects, and prognoses[7]. This not only affects our ability to explore the pathogenesis of OCD but also presents challenges in selecting effective treatment options for patients[8].

In clinical practice, most patients with OCD often exhibit both obsessive thinking and compulsive actions[9]. The classification method in the International Classification of Diseases (ICD) 10th revision (ICD-10) cannot be used to select effective clinical treatment plans and has been removed from ICD-11. Thus, there is currently no unified clinical classification or evaluation standard for OCD. Although many studies have explored the symptom dimensions of OCD and attempted to lay a foundation for its classification, no conclusions have been reached.

This study aimed to discuss the symptom dimensions of OCD in China. We attempted to explore the symptom dimensions of Chinese patients with OCD through a systematic cluster analysis of the categories of the Yale-Brown Obsessive-Compulsive Scale Checklist (Y-BOCS-CL), compare the results obtained with those of a previous study, and explore the relationships between the main clinical characteristics of patients and the symptom dimensions obtained in our study.

[1]Capital Medical University, Beijing Anding Hospital, National Clinical Research Center for Mental Disorders and National Center for Mental Disorders, Beijing Key Laboratory of Mental Disorders – Beijing, China.
[2]Capital Medical University, Advanced Innovation Center for Human Brain Protection – Beijing, China.
[3]Weifang People's Hospital, Department of Clinical Psychology – Weifang, China.
*Corresponding author: lizhj8@ccmu.edu.cn

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METHODS

Participants

A total of 263 outpatients with OCD were recruited from Beijing Anding Hospital, Capital Medical University, and Weifang People's Hospital between September 2017 and September 2021 who met the diagnostic criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-4)[10]. Diagnosis was made by attending psychiatrists with significant experience in diagnostic interviews using the Mini International Neuropsychiatric Interview (MINI)[11]. The severity of illness was determined using the Y-BOCS[12]. Patients were included if they were between the ages of 18 and 65 years, had a score ≥7 on the Y-BOCS, and had a cultural level of junior high school or above. Exclusion criteria included schizophrenia, bipolar disorder, mental retardation, OCD occurring exclusively in the context of depression, a history of organic brain disease, major physical disease, drug dependence, and psychoactive substance use. This study was approved by the Research Ethics Committee of Beijing Anding Hospital, Capital Medical University (201941FS-2).

All participant symptoms were assessed using the Y-BOCS and Y-BOCS SC. The Y-BOCS was used to evaluate the severity of obsessive-compulsive symptoms and has satisfactory inter-rater reliability and construct validity. The Y-BOCS includes the obsessive, compulsive, and total scale scores, with a higher score indicating more severe symptoms. A total Y-BOCS score of <16 is classified as mild or subclinical; 16–22 is classified as moderate; 23–31 is classified as severe; and >31 is classified as extremely severe[13,14].

The Y-BOCS SC is a semistructured interview outline for the Y-BOCS, which comprises eight categories of obsessions (aggressive, contamination, sexual, hoarding/saving, religious, symmetry or exactness, somatic, and miscellaneous) and seven categories of compulsions (cleaning/washing, checking, repeating, counting, ordering/arranging, hoarding/saving, and miscellaneous). With a total of 68 items, the Y-BOCS SC has been extensively used in research and clinical settings for the past two decades and is generally assumed to possess good reliability and validity[15]. However, the two categories related to hoarding, each containing two items, were not evaluated, as hoarding disorder is regarded as an independent diagnosis of OCD in the DSM-5[16]. Likewise, two miscellaneous categories, which encompass 17 items and exhibit high heterogeneity and low mutual consistency, were excluded from this study. As different patients exhibit different manifestations, it is not feasible to conduct unified data processing. Consequently, this study eliminated the two hoarding categories and two miscellaneous categories of the Y-BOCS, leaving 11 categories to process the data.

Statistical analysis

All collected data were entered into the SPSS software version 26.0 for Windows (IBM/SPSS Inc., New York, USA). Pearson analysis was conducted to explore the relationships between the main clinical characteristics and symptom dimension scores in our sample. Statistical significance was assumed at $p<0.05$.

RESULTS

Demographic and clinical findings

In total, 263 patients were included in the study, comprising 142 males (54.0%) and 121 females (46.0%). The average age of the participants was 32.09±8.32 years (18–64 years), with a mean age of onset of 21.94±6.81 years. The duration of the illness ranged from 1 month to 37 years, with an average of 6.76±6.63 years.

Approximately 72.62% (191/263) of patients were treated with serotonin reuptake inhibitors (SRIs). The use of benzodiazepines, such as diazepam, lorazepam, and oxazepam, was uncommon (36/263, 13.69%). A total of 10.27% (27/263) of participants received low doses of atypical antipsychotics, including risperidone, olanzapine, aripiprazole, and quetiapine. Cognitive-behavior therapy (CBT) was administered to a small proportion of the participants (33/263, 12.55%). Detailed clinical and demographic data are presented in Table 1.

System cluster analysis findings

Figure 1 presents the cluster analysis outcomes after scoring 11 Y-BOCS SC categories. The 11 Y-BOCS SC categories were divided into two, three, four, or five symptom dimensions. Combining domestic and foreign research results on OCD symptom content classification, this study surveyed 19 senior psychiatrists specialized in OCD to determine the content classification of OCD symptoms. Among the experts, 53% (10) supported the four-dimensional classification of OCD symptom content: symmetry and precision, contamination and cleanliness, aggressive examination, and taboo thinking. This study adopted four symptom dimensions (Figure 1).

Findings from Pearson analysis

Pearson analysis revealed a significant relationship between years of education and Dimension 1 (symmetry precision dimension, r=-0.13) (Table 2). Additionally, there were significant relationships between compulsive score, total Y-BOCS

Table 1. Demographic and clinical characteristics of participants.

Clinical variables	OCD (n=263) Mean±SD/frequency
Age (years)	32.09±8.32
Gender (male/female)	142/121
Marital status (single/married)	141/122
Years of education	14.20±2.79
Ethnicity (Han/other)	246/17
Religion (yes/no)	20/243
Family history (negative/positive)	228/35
Age at onset (years)	21.94±6.81
Duration of illness (years)	6.76±6.63
Y-BOCS total score	22.17±6.47
Obsession score	11.49±4.55
Compulsion score	10.68±4.89
Currently on SRI (yes/no)	191/72
Any benzodiazepine (yes/no)	36/227
Any antipsychotic (yes/no)	27/236
On CBT (yes/no)	33/230

OCD: obsessive-compulsive disorder; Y-BOCS: Yale-Brown Obsessive Compulsive Scale; SRI: serotonin reuptake inhibitors; CBT: cognitive-behavior therapy.

score, and Dimension 2 (contamination cleaning dimension, r=0.19, and r=0.23, respectively) and Dimension 3 (aggression examination dimension, r=0.17, and r=0.17, respectively). Furthermore, age, age at onset, obsessive score, and compulsive score showed significant relationships with Dimension 4 (taboo-thinking dimension, r=-0.12, r=-0.12, r=0.25, and r=-0.16, respectively).

DISCUSSION

In this study, system cluster analysis was used to analyze the symptoms of 263 patients with OCD. OCD symptoms in this study were divided into four dimensions: (1) symmetry precision dimension (this dimension included symmetry or exactness obsession and ordering/arranging compulsion); (2) contamination cleaning dimension (this dimension included contamination obsession, cleaning/washing compulsion, and somatic obsession); (3) aggression examination dimension (this dimension included repeating compulsion, counting

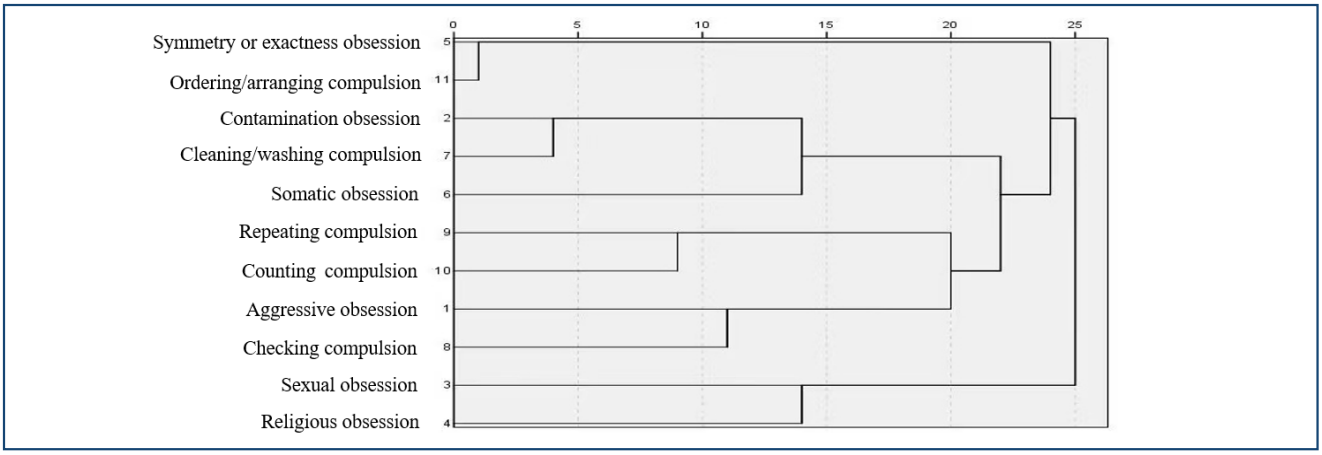

Figure 1. System cluster analysis results of the 11 Yale-Brown Obsessive-Compulsive Scale Symptoms Checklist categories.

Table 2. Correlation between the main clinical characteristics and four symptom dimensions.

Variables	Dimension 1		Dimension 2		Dimension 3		Dimension 4	
	r	P	r	P	r	P	r	P
Gender	-0.04	0.57	0.08	0.22	-0.05	0.42	-0.05	0.45
Age	-0.08	0.21	<0.01	0.99	0.08	0.21	-0.12*	0.046
Age of onset	-0.04	0.57	0.07	0.25	0.01	0.83	-0.12*	0.044
Disease duration	-0.05	0.43	-0.10	0.10	0.05	0.39	-0.04	0.50
Years of education	-0.13*	0.04	<0.01	0.96	-0.09	0.17	-0.08	0.17
Family history	-0.07	0.24	-0.08	0.19	-0.01	0.86	-0.06	0.35
Obsessive score	0.07	0.24	0.11	0.09	0.06	0.37	0.25*	<0.01
Compulsive score	0.03	0.60	0.19*	<0.01	0.17*	<0.01	-0.16*	<0.01
Total Y-BOCS score	0.06	0.35	0.23*	<0.01	0.17*	<0.01	0.66	0.29

*p≤0.05 was considered to show a significant association. Dimension 1: symmetry precision dimension; Dimension 2: contamination cleaning dimension; Dimension 3: aggression examination dimension; and Dimension 4: taboo thinking dimension.

compulsion, aggressive obsession, and checking compulsion); and (4) taboo thinking dimension (this dimension included sexual and religious obsessions).

Our study's findings were consistent with previous research, which identified the same main symptom dimensions in OCD. For instance, Pinto et al.[17] and another study found five factors, namely, symmetry/ordering, hoarding, doubt/checking, contamination/cleaning, and taboo thoughts[17]. While our study excluded hoarding symptoms due to their separation from OCD as an independent diagnosis in DSM-5, the remaining dimensions were consistent. However, discrepancies exist between domestic and international studies, with classification methods ranging from 3 to 7. Although numerous studies have been conducted on OCD symptom dimensions, a definitive conclusion has not yet been reached.

Differing classification methods in OCD studies may be due to data processing techniques and symptom selection. Most studies used factor analysis, which may overlook some symptoms, while our study utilized system cluster analysis, which provides a more comprehensive understanding of symptom dimensions. Therefore, cluster analysis can lead to a more comprehensive understanding and analysis of the dimensions of obsessive-compulsive symptoms, a view that has also been confirmed by Cameron et al.[18]. Additionally, cultural and sample size differences may also contribute to discrepancies. Despite these differences, our study found consistent symptom dimensions in Western countries, suggesting stability across regions and sociocultural contexts.

The study found a significant correlation between years of education and Dimension 1, suggesting that patients with symmetry precision symptoms had fewer years of education. This aligns with previous studies that reported an association between symmetrical symptom groups and years of education[19]. Limited research is available on this phenomenon, indicating the need for further investigation.

The study found positive correlations between compulsive score, total Y-BOCS score, and Dimensions 2 and 3, indicating that patients with contamination or attack fears have more compulsions for repeated cleaning and examination. These findings align with another study that identified cleaning/washing, repeating/redoing, and checking as the most common types of compulsion[20]. Dimension 4 symptoms, primarily related to taboos surrounding sex, were more likely in younger individuals with earlier onset, higher obsessiveness, and lower compulsiveness. Lower religiosity in Chinese OCD patients may be linked to psychological factors in early adulthood.

This study had several limitations. First, the sample was drawn from only two locations in China, which may not be representative of the patients nationwide. Second, the coronavirus disease 2019 (COVID-19) pandemic may have affected the patients' symptoms; thus, further research is necessary to confirm these findings. Finally, this cross-sectional study lacked follow-up data, which could potentially provide useful insights into the evolution of symptoms and validation of the current results.

CONCLUSION

Our findings have revealed that the symptoms of OCD in Chinese patients are multi-dimensional. The four symptom dimensions identified in this study were consistent with those reported in previous studies, suggesting that OCD symptoms are similar across different regions. However, each dimension showed distinct clinical characteristics, which may indicate different pathogenic mechanisms underlying OCD. Our research provides a basis for future studies to explore the symptom dimensions, diagnosis, and pathogenesis of OCD.

AUTHORS' CONTRIBUTIONS

XL: Conceptualization, Methodology, Writing – review & editing. **YZ:** Conceptualization, Data curation, Writing – original draft. **PW:** Data curation, Investigation, Software, Validation, Visualization, Writing – original draft. **XY:** Conceptualization, Supervision, Writing – review & editing. **ZL:** Conceptualization, Methodology, Software, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Investigation of rational drug use behaviors and knowledge levels of older individuals: a cross-sectional study

İpek Bakimli[1*], Emel Tuğrul[2]

SUMMARY

OBJECTIVE: The aim of this study was to examine older individuals' rational drug use behavior, their knowledge of rational drug use, and the factors affecting it.

METHODS: This study was conducted cross-sectionally with 440 patients aged 65 years who received inpatient treatment in internal medicine and surgery clinics between October 2021 and November 2022 using a Rational Drug Use Scale and rational drug use behavior questions.

RESULTS: The findings showed that the mean age of older adults was 72.56±5.84 years, and 51.8% were men. It was determined that 79.1% of the older adults did not check their expiration date before using the medicines, and 85.9% of them retained the remaining medicines after treatment. Results indicated that 77.3% of older adults knew less about rational drug use. Additionally, a significant difference was observed between older adults' marital status, educational status, possession of outdated drugs at home, self-use of antibiotics without examination, and mean score on the Rational Drug Use Scale ($p<0.05$).

CONCLUSION: The results showed that the rational drug use knowledge level of older adults was low and that there were differences in the knowledge levels of rational drug use according to certain behaviors and factors.

KEYWORDS: Rational drug. Older adults. Behavior.

INTRODUCTION

Rational drug use (RDU) is a rule that must be followed for patients to take drugs according to their needs, the right dosage, sufficient time intervals, and the least cost to themselves and society[1]. Although more than 50% of drugs are improperly prescribed, distributed, and sold worldwide, 50% of patients cannot take medications correctly, and approximately one-third of the global population cannot access essential drugs[2].

The proportion of the older population in Turkey was 5.7% in 2000, which is expected to increase to 9.7% in 2021[3]. With aging, the number of chronic diseases increases; therefore, the number of older individuals using drugs is increasing. A study conducted with 300 older individuals reported that 58.3% of the individuals used four or more than four drugs, and 72.7% of these drugs were cardiovascular drugs[4]. Another study of 171 older individuals stated that 42.69% of the older adults used five or more drugs, and 94.5% experienced drug-related side effects[5].

In one study, the prevalence of irrational drug use (IDU) was 44.2%, and the most commonly used drugs in older adults were analgesics[6]. Another study of 190 older individuals found a positive correlation between IDU use and polypharmacy, polypathology, and hypertension[7].

Investigating risk factors for drug use in older adults and early intervention with controllable factors can reduce the risk of death[8]. The determination of RDU levels and factors affecting older individuals worldwide and in our country is essential for the health-care system. This study aimed to determine drug use behaviors and knowledge levels in an elderly population. The study will provide scientific contributions to determining the roadmap for RDU and the factors affecting IDU in older individuals. Furthermore, in line with the scientific data obtained at the end of the study, it is predicted that nurses and other health professionals responsible for rational drug management will contribute to taking the necessary precautions regarding the issue in older individuals and the spread of these precautions.

METHODS

This cross-sectional study was conducted in the internal medicine and surgery clinics of the Aydın Adnan Menderes University Hospital between October 2021 and November 2022.

The number of individuals included in the study sample was determined using the sample calculation method of the unknown universe. Accordingly, the minimum number of individuals to be

[1]Manisa Merkezefendi State Hospital, Anesthesia Intensive Care Unit – Manisa, Turkey.
[2]Aydın Adnan Menderes University, Nursing Faculty, Department of Fundamentals of Nursing – Aydın, Turkey.
*Corresponding author: bakimliipek@gmail.com

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sampled was calculated based on n=unknown, p=0.50, q=0.50, and t=1.96 (α=0.05) values and found to be 384. Considering the possible loss of cases (approximately 10%) in the study sample, 440 older individuals (228 women and 212 men) were included in the sample using a random sampling method (convenience sampling). Patients with memory and hearing problems and those diagnosed with psychiatric diseases were excluded from the study.

The study data were collected through face-to-face interviews in clinics where the patients received treatment between 08:00 and 17:00 on weekdays. Questions in the form of essential features in which sociodemographic characteristics were questioned, a questionnaire questioning RDU behaviors, and the Rational Drug Use Scale (RDUS) were used to determine the knowledge levels of rational drug use. Data collection lasted for an average of 20–30 min for each participant.

Question form: It comprised 22 questions based on a literature review on rational drug use behaviors in elderly individuals[9-11].

The RDUS is a 21-item scale used to determine individuals' RDU knowledge levels[12]. For each situation on the scale, the numbers correspond to statements on the scale; they are evaluated as 0-no, 1-do not know, and 2-yes. As the scores obtained from the scale increased, the knowledge of RDU increased. The cutoff point for the scale was 34, and knowledge of RDU with a score of 35 or above was considered high. Cronbach's alpha coefficient of the scale was 0.789. In this study, Cronbach's alpha of the scale was 0.495.

The SPSS Windows software version 25.0 was used to evaluate the data. The data analysis used the percentage distribution and descriptive statistics to define sociodemographic characteristics. The data conformity to the normal distribution was checked using the Kolmogorov-Smirnov test of normality (RDUS: K-S:0.120, p=0.000). Since the data did not show a normal distribution, the Mann-Whitney U, Kruskal-Wallis, and Bonferroni tests were used. Statistical significance was set at $p<0.05$.

RESULTS

The mean age of 440 older people included in the study was 72.56±5.84 years; 51.8% were men, 23.9% were hospitalized in internal medicine clinics, 75.7% were married, 54.1% were primary school graduates, and 48.6% were stay-at-home parents. It was found that 91.6% had an income equal to their expenses, and 75.7% lived with their spouses. Among the older participants, 78.6% had a chronic disease, 45.2% had hypertension, 26.6% had diabetes mellitus, 13.6% had heart disease, 5.2% had chronic kidney failure, 16.8% had chronic obstructive pulmonary disease, 8.6% had asthma or bronchitis, 5.0% had epilepsy, and 10.9% had prostate disease. It was determined that 89.5% of the participants were 1 km or closer to their health institution; 85.9% used regular drugs; 29.4% used three drugs; 21.7% used antihypertensive drugs; and 12.6% used diabetic drugs. Behaviors of older adults regarding rational drug use are presented in Table 1.

Distribution of Rational Drug Use Scale mean and median scores and knowledge levels of older adults are presented in Table 2. There was a significant difference ($p<0.05$) between marital status, educational level, control of the expiration date of the drugs, possession of expired drugs at home, self-use of antibiotics without examination, and RDUS score averages of older adults. Additionally, the RDUS score average of older adults who did not control the expiration date of the drugs was lower than the average score of older adults who did. It was observed that the mean RDUS score of older adults who did not use antibiotics themselves was higher than that of those who used them (Table 3).

DISCUSSION

Most older participants in our study used drugs regularly, and 29.4% used three drugs. The most commonly used drugs are antihypertensive, diabetic, and heart drugs. In a health center study, it was found that older adults mostly used drugs from the cardiovascular (53.5%) and endocrine (9.5%) systems[13]. Chronic diseases that are frequently observed in older adults have led to the use of many drugs. A study on older patients discharged from the hospital reported that 92.1% of older adults were prescribed between 3 and 19 drugs for use after discharge[14]. The use of many drugs by older adults indicates that they are a special group that should be given more importance in RDU.

In our study, most older patients took medication after consulting a physician when sick. In a study conducted by Yılmaz et al.[15] on older individuals, it was reported that 54.1% of the participants applied to a physician when sick, and 29.1% took medication by applying to a pharmacist. This result shows that older individuals try to solve their health problems by getting help from a health institution as they should; this can be considered positive behavior. Almost all older adults did not use antibiotics independently without an examination because of flu, cold, and flu complaints. Older adults commonly use painkillers, stomach protectors, cold drugs, and vitamins without a prescription[5,15-17]. The absence of over-the-counter antibiotic sales in our country has created a favorable situation by limiting the uncontrolled use of antibiotics. It was determined that a tiny proportion of elderly individuals used drugs recommended by their neighbors or relatives. Studies conducted in our country have reported that the rate of drug use based on the advice of neighbors, family, and friends is low among older adults[5,16,17].

In this study, most older individuals did not check their expiration date before drug use. Most participants kept the drugs in

Table 1. Behaviors of older adults regarding rational drug use (n=440).

Behaviors	n	%
Using the drug recommended by a neighbor or a relative		
Yes	32	7.3
No	408	92.7
Persons who received information on how to use regularly used drugs		
Physician	366	83.2
Pharmacist	74	16.8
Nurse	0	0
Getting information about the side effects of the drugs used		
Yes	5	1.1
No	435	98.9
Reading the leaflet before using the medicines		
Yes	47	10.7
No	393	89.3
Status of remaining drugs after treatment		
Save it in case you need it again	378	85.9
Giving to those who need it	8	1.8
Castaway	38	8.6
Other	2	0.5
Take to pharmacy	14	3.2
Paying attention to the storage conditions of medicines at home		
Yes	395	89.8
No	45	10.2
Storing medicines in their own box		
Yes	409	93.0
No	31	7.0
The medicine storage place at home		
A special drawer	251	57.0
A special locker	71	16.1
Medicine cabinet	14	3.2
Freezer	92	20.9
Other	12	2.7
Buying medicine from the pharmacy without a physician's examination		
Yes	101	23.0
No	339	77.0
Experiencing side effects of any drug used		
Yes	32	7.3
No	408	92.7
What to do in case of side effects (n=32)		
Contacting a physician	30	93.75
Contacting a pharmacist	2	6.25
Taking the medication with television or media promotion without consulting a physician		
Yes	3	0.7
No	437	99.3

their boxes and paid attention to their storage conditions. In the literature, most older adults who know the storage conditions of drugs[18,19] store drugs at room temperature, and individuals between the ages of 60 and 65 years prefer refrigerators[16].

In our study, most older adults (91.4%) used prescribed drugs. However, the literature has stated that older adults use their drugs for different durations or in different ways than desired[8,15,18-20].

RDU knowledge levels were evaluated using the scale used in the study. Accordingly, it was determined that participants' RDU knowledge level was low. In studies conducted using the same scale, different results were obtained regarding knowledge levels in older individuals[21-24]. The scale included topics such as the method of using drugs, their side effects, the use of multiple drugs, the duration of treatment, and what to do in case of undesirable effects. Accordingly, it can be said that due to the lack of knowledge among older adults about drugs, these issues should be addressed. It is estimated that the emergence of progressive memory problems with increased multimorbidity and multiple drug use in aging individuals may affect their RDU knowledge level. It is predicted that the knowledge level regarding RDU can be increased by educating older adults.

In our study, among the factors affecting the RDU knowledge level of older individuals, increasing the education level of individuals and being married stood out as factors that positively affected the average score of rational drug use knowledge level. The mean score of the illiterate elderly was lower than that of those with other educational levels. Education levels can affect individuals' reading comprehension levels. Therefore, it can be seen that these individuals have higher RDU knowledge levels. In the studies reviewed in the literature, it was observed that individuals' RDU behaviors and knowledge levels were affected by many variables. Hence, generalizations could not be made[16,25].

CONCLUSION

In our study, although most of the RDU behavioral data of older people were positive, their knowledge level of RDU was low. The observed situation can be attributed to older individuals maintaining their established behaviors, which align with their long-standing habits. Furthermore, the decline in learning skills and memory abilities that accompany aging significantly affects their knowledge levels.

Continuous training of individuals, families, and health professionals in light of the information in our study is recommended. Governments should initiate activities to improve the level of knowledge of RDU, create a standardized RDU chain in health institutions, and prepare drug prospectuses more simply and understandably.

Table 2. Distribution of Rational Drug Use Scale mean and median scores and knowledge levels of older adults (n=440).

Scale	mean±SD	Median (QS)	min–max
RDUS	32.52±2.93	32.00 (3.00)	21.00–41.00
		n	%
RDU level of knowledge			
Low level of knowledge (≤34 points)		340	77.3
High level of knowledge (>35 points)		100	22.7

QS: quarterly span; SD: standard deviation; RDUS: Rational Drug Use Scale.

Table 3. Comparison of some descriptive characteristics and rational drug use behaviors of older adults and Rational Drug Use Scale mean or median scores (n=440).

Variables	n	mean±SD	Median (QS)	Statistics
Marital status				
Married	333	32.78±2.67	33.00 (3.00)	z: -2.573
Single	107	31.69±3.51	32.00 (4.00)	p: 0.010*
Educational levels				
Illiterate (a)	18	29.22±3.81	28.50 (3.75)	
Literate (b)	21	30.09±4.14	32.00 (6.50)	x^2: 26.823
Primary school (c)	238	32.68±2.59	33.00 (3.00)	p: 0.000*
Middle school (d)	136	32.91±2.54	33.00 (3.75)	a<c=d=e**
High school/university (e)	27	33.22±3.75	33.00 (6.00)	
Remembering the time and dose while using the drug				
Yes	396	32.70±2.66	33.00 (3.00)	z: -2.328
No	44	30.90±4.46	32.00 (7.75)	p: 0.020*
Checking the expiration date before using medication				
Yes	92	33.46±3.55	33.00(4.00)	z: -2.932
No	348	32.27±2.70	32.00(3.00)	p: 0.003*
Presence of expired medication in the home				
Yes (a)	12	33.91±3.20	33.00(4.75)	x^2: 9.433
No (b)	104	33.26±3.40	33.00(4.00)	p: 0.009*
I don't know (c)	324	32.23±2.71	32.00(3.00)	b>c***
Using antibiotics without examination				
Yes	10	28.50±2.95	28.00(4.50)	z: -3.804
No	430	32.61±2.87	33.00(3.00)	p: 0.000*

*p<0.05, ***p-value obtained as a result of Bonferroni correction p<0.0167, QS: quadrants span; SD: standard deviation; z: Mann-Whitney U test; x^2: Kruskal-Wallis test.

ETHICAL ASPECTS

This study was approved by the Aydın Adnan Menderes University Faculty of Nursing Noninterventional Clinical Research Ethics Committee (No. 50107718-050.99) and official permission from the Aydın Adnan Menderes University Hospital before starting the study. Additionally, verbal consent was obtained from the individuals included in the study before data collection. This study has been conducted in accordance with the principles set forth in the Declaration of Helsinki.

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PATIENT CONSENT STATEMENT

A verbal consent was obtained from the conscious patients. The participants were informed of the purpose of the study as well as that participation was voluntary and the data would remain confidential.

AUTHORS' CONTRIBUTIONS

İB: Conceptualization, Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **ET:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Antioxidant effect of *Rosa pimpinellifolia* L. fruit extract on cholestatic liver injury: an experimental study

Mahmut Kaan Demircioglu[1*], Zeynep Gul Demircioglu[2], Ozlem Cakir[3], Karolin Yanar[4], Muveddet Banu Yilmaz Ozguven[5], Pinar Atukeren[4], Osman Bilgin Gulcicek[6], Bulent Citgez[2], Pinar Yazici[2]

SUMMARY

BACKGROUND: Antioxidants have been considered a rational curative strategy to prevent and cure liver diseases involving oxidative stress. An acute obstructive jaundice rat model was established to investigate the ***in vivo*** hepatoprotective efficacy of *Rosa pimpinellifolia* L.

METHODS: The experimental jaundice model was performed by binding the main bile duct in 25 male Sprague-Dawley rats. All rats were randomly divided into five groups: first group: laparotomy-sham-only, second group: biliary tract binding (control), and third, fourth, and fifth groups: treatment groups with 250, 500, and 750 mg/kg fruit extracts daily, respectively.

RESULTS: Considering dosage, although there was no significant therapeutic effect in the 250 mg/kg of *Rosa pimpinellifolia* L. group, the best results were found in the 500 mg/kg dose group, while results in the 750 mg/kg dose group showed consistent correlation with proinflammatory response. With regard to biochemical parameters, lipid hydroperoxide level in the rat serum and liver tissue was significantly decreased in all treatment groups. Amadori products, which are one of the early markers of glycol-oxidative stress, showed statistical significance in the treatment.

CONCLUSION: It was revealed that the antioxidant effect of *Rosa pimpinellifolia* L. was more prominent in the early stages of hepatic injury secondary to oxidative stress.

KEYWORDS: Cholestasis. Liver. Antioxidants. *Rosaceae*.

INTRODUCTION

Antioxidants are chemicals that help organisms to lessen or eliminate oxidative stress caused by free radicals. Many wild fruits are usually considered a good source of antioxidants and have been used as natural therapeutic agents due to their bioactive phenolic compounds[1]. One of the most important wild fruit groups is the *Rosaceae* family which includes thousands of species, and some of the vital phytochemicals and antioxidants in the fruits of this family have potential health benefits[2].

Rosa pimpinellifolia L. fruit that belongs to the Rosaceae family is an endemic member of the Rosaceae family growing in the (Bayburt Province) East of Turkey[3].

Recently, Ergen et al., showed the antioxidant activity of *R. pimpinellifolia* L. *in vitro*[4]. However, *in vivo* antioxidant activity has not yet been confirmed. Therefore, this study was designed to investigate the potential antioxidant activity of *R. pimpinellifolia* L. fruit extract on an experimental model of liver injury.

METHODS

Animals and preparations

A total of 25 adult male Sprague-Dawley rats (age, 6–8 weeks; weight, 290±30 g) were housed at a constant temperature (22±1°C), with 50% relative humidity and a 12-h light/dark cycle. The rats had access to food including 21% protein and autoclaved water *ad libitum*. All animal procedures were approved by the Animal Experiments Ethics Committee.

[1]Basaksehir Cam and Sakura City Hospital, Department of Surgical Oncology – İstanbul, Turkey.
[2]Sisli Hamidiye Etfal Training and Research Hospital, Department of General Surgery – İstanbul, Turkey.
[3]Bayburt University, Faculty of Engineering, Department of Food Engineering – Bayburt, Turkey.
[4]Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Department of Biochemistry – İstanbul, Turkey.
[5]Sisli Hamidiye Etfal Training and Research Hospital, Department of Pathology – İstanbul, Turkey.
[6]Bagcilar Training and Research Hospital, Department of General Surgery – İstanbul, Turkey.
*Corresponding author: mahmutkaandemircioglu@gmail.com

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Procedures were performed under general anesthesia. Anesthesia was provided with ketamine and xylazine (10 mg/kg xylazine and 50 mg/kg ketamine) administered intraperitoneally.

Bile duct surgery was performed as previously reported by Criado et al.[5]. The common bile duct (CBD) was tied with 5/0 silk thread from two places to create obstructive jaundice by clearing it from surrounding tissues.

Groups and treatments

The rats were randomly divided into five groups, with five animals in each group:

Group 1: Animals were performed only laparotomy and treated orally with normal saline (0.9%) for 10 days.

Group 2: Control animals were performed laparotomy and CBD ligation and treated orally with normal saline (0.9%) for 10 days.

Group 3: After CBD ligation, oral *R. pimpinellifolia* L. fruit extract 250 mg/kg for 10 days.

Group 4: After CBD ligation, oral *R. pimpinellifolia* L. fruit extract 500 mg/kg for 10 days.

Group 5: After CBD ligation, oral *R. pimpinellifolia* L. fruit extract 750 mg/kg for 10 days.

Rosa pimpinellifolia L. fruits were collected from Bayburt (Eastern Turkey). The collected seeds were dried in an oven and pounded in a porcelain mortar. Notably, 20 g of sample taken from the powdered sample was added to the 100 mL ethanol (EtOH)-water (50/50 mL) mixture. The mixture was extracted in a water bath under a constant temperature of 40°C for 48 h. Then, the extract was filtered and, to remove EtOH, it was subjected to evaporation at 40°C using an evaporator. The extract was frozen at -80°C and dried in a lyophilizer, and *R. pimpinellifolia* L. 50% EtOH and water extract was obtained and stored at -20°C.

The experiment was completed. Before sacrification, a circulating blood sample (7 mL) was obtained by cardiac puncture. A portion of the left liver lobe was taken for histochemical and biochemical analyses.

Laboratory analysis

Serum biochemical parameter levels such as aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and total bilirubin were analyzed with the colorimetric method.

The liver tissues were homogenized in a buffer [phosphate buffer (pH 7.4)+0.1% digitonin] with a ball-bearing homogenizer and 10% homogenates were prepared.

Myeloperoxidase activity and nitrotyrosine level were calculated by ELISA kit; lipid hydroperoxide (LOOH) levels were calculated by extinction coefficient; malondialdehyde (MDA) total thiol levels and Amadori products were calculated by extinction coefficient; advanced glycation end products (AGE) were calculated by the spectrofluorimetric method; and ferric ion-reducing antioxidant power (FRAP) was calculated with the calibration graph.

Histopathological assessment

The liver specimen was fixed in 10% formalin solution and embedded into paraffin blocks. The blocks were cut using microtome to 5 µm thickness. Thereafter, the sections were stained by hematoxylin-eosin (H&E) as well as Masson trichrome. Tissue slides were visualized under a light microscope. The same pathologist with at least 10 years of experience made all evaluations in liver pathology.

Statistical analysis

Statistical analyses were performed using the SPSS software version 25.0 (IBM SPSS Statistics for Windows, Version 25.0. IBM Corp. Released 2017. Armonk, NY: Chicago, IL, USA).

Quantitative data were expressed as the mean±standard deviation. After determining whether the parameters were compatible with the normal distribution or not using the Shapiro-Wilk test, one-way analysis of variance (ANOVA) was applied for parametric tests with normal distribution using the Bonferroni test for *post hoc* comparisons when significance was determined by ANOVA. The Kruskal-Wallis test was used with nonparametric distribution. Values with $p<0.05$ were considered statistically significant. A *post hoc* test was used for paired group comparisons in parameters that were significant.

RESULTS

Biochemical results

Rat serum liver function tests (aspartate transaminase/alanine transaminase/total bilirubin/gamma-glutamyltransferase/alkaline phosphatase)

By examining liver function tests from rat serums, a comparative analysis between the control and treatment groups (Groups 2 and 3–5) and the sham group (Group 1) resulted in a significant difference ($p<0.05$) which was compatible with the cholestatic liver injury. However, in treatment groups compared with the control group, the best results—similar AST levels to the sham surgery group—considering AST were found in Group 4 (500 mg/kg). Although the alterations in ALT levels were unremarkable, better results were observed in Groups 4 and 5.

Rat serum oxidative damage parameters

Lipid peroxidation and glycoxidative stress parameters

Lipid peroxidation (LOOH, MDA) and glycoxidative stress (AGE, Amadori) parameters were statistically significant in the control group (Group 2) (Table 1). When the extracted groups were compared with the control group, lipid peroxidation and glycoxidative stress parameters were found to be statistically significant and low.

Other antioxidant parameters

Considering the efficacy of treatment in terms of parameters other than FRAP in rat serum, no significant difference was found.

Rat liver homogenate oxidative stress parameters

Protein oxidation parameters

As indicated by the PCO, DT, and KYN (protein carbonyl groups, DT: dytyrosine, KYN: kynurenine) values in rat liver tissue, there were findings of oxidative damage in both control and therapeutic groups, and no positive effect was found between the groups in terms of different dosages.

Lipid peroxidation and glycoxidative stress parameters

LOOH level in rat liver tissue increased significantly in the control group (Group 2), and a significant decrease was observed in the groups that were given the extract (p=0.014). There was no significant change in MDA levels in terms of treatment efficacy.

A statistical significance was achieved with the increase of Amadori, one of the early markers of glycoxidative stress, in the control group (Group 2), while approaching the level of the sham group in the groups that were given the extract (p<0.001). On the contrary, no significant difference was found in the AGE level, which indicates the advanced stage of glycoxidative stress (Table 2).

Other antioxidant parameters

Among other antioxidant parameters studied from rat liver tissue, T-SH levels were not affected. In addition, although there is a regression in FRAP levels in the extracted groups compared with the sham group, there is an increase in the antioxidant parameters when evaluated compared with the control group. Cu and Zn SOD levels increased in the groups that were given the extract compared with the control group. The effect of the increase in antioxidant levels in the liver tissue among the groups that were given the extract was highest in the group (Group 4), in which the extract was administered at a dose of 500 mg/kg (Table 3).

Table 1. Mean values of oxidative damage parameter tests (lipid peroxidation and glycoxidative) in serum samples of rats between groups.

	LOOH* (µmol/mL)	MDA* (µmol/mL)	AGE** (FU/mg protein)	Amadori** (nmol/mg protein)
Group 1	1.26±0.02	2.01±0.09	1039±143	82199±11392
Group 2	1.47±0.8	2.09±0.11	2581±360	204193±28509
Group 3	1.29±0.06	2.20±0.11	1233±617	97547±48841
Group 4	1.22±0.06	2.12±0.18	1411±686	111677±54325
Group 5	1.2±0.07	1.93±0.76	1110±607	87816±48036
p-value	***<0.001***	***0.025***	***0.028***	***0.027***

*Parametric distribution; **non-parametric distribution. LOOH: lipid hydroperoxide; MDA: malondialdehyde; AGE: advanced glycation end products. Bold italics denote statistically significant p-value.

Table 2. Mean values of oxidative damage parameter tests (lipid peroxidation and glycoxidative) in liver tissue of rats between groups.

	LOOH** (µmol/mg protein)	MDA** (µmol/mg protein)	AGE* (FU/mg protein)	Amadori* (nmol/mg protein)
Group 1	0.9±0.4	4±0.2	3156±567	47±19
Group 2	2.5±0.2	5.7±1.1	3608±1508	94±13
Group 3	0.8±0.1	4.6±1.01	3092±134	45±13
Group 4	0.7±0.2	6±0.5	4490±848	40±16
Group 5	0.7±0.1	5.5±0.9	3032±759	39±14
p-value	***0.014***	0.806	0.066	***<0.001***

*Parametric distribution; **non-parametric distribution. LOOH: lipid hydroperoxide; MDA: malondialdehyde; AGE: advanced glycation end products. Bold italics denote statistically significant p-value.

Table 3. Average values of other antioxidant levels in liver tissue of rats between groups.

	T-SH* (nmol/mg protein)	FRAP** (µmol/mg protein)	Cu, Zn SOD* (U/mg protein)
Group 1	10±1.1	2303±15215	31.7±20
Group 2	12.5±3.2	593±134	4.1±1.8
Group 3	13.5±2.6	1627±805	24±52
Group 4	14±2.4	1734±599	57±15
Group 5	13.7±1.7	1240±420	33±12
p-value	***0.089***	***0.017***	***0.001***

*Parametric distribution; **non-parametric distribution. T-SH: total thiol groups; FRAP: ferric ion-reducing antioxidant power; Cu, Zn SOD: copper, zinc superoxide dismutase. Bold italics denote statistically significant p-value.

Histopathological results

Except for the sham group, similar rates of ductular proliferation, inflammatory cell infiltration in the ducts, acidophilic necrosis, coagulation necrosis, and fibrosis were detected in all groups. However, when the groups were compared, none of the parameters were found to be statistically significant.

Although not statistically significant, increased mitotic activity was detected in the groups that were given the extract. Although it was not reflected in the statistical data, there was an increase in the number of mitoses correlated with the increase in the dose of the extract administered.

DISCUSSION

The final results showed that the jaundice model created in rats was successful, and the best results considering antioxidant activity were achieved in the 500 mg/kg group. In the low dosage (250 mg/kg) group, a significant therapeutic effect was not observed, while the proinflammatory effect was more prominent in the high dosage (750 mg/kg) group.

The binding of the CBD in rats produces similar changes to those in human biliary cirrhosis[6]. In a study, it was determined that ALT, AST, total bilirubin, GGT, and ALP levels in plasma increased 10 days after the main bile duct was disconnected, causing significant cholestatic liver damage[7]. Likewise, we determined the study duration as 10 days. All values (AST, ALT, T.Bil, and GGT) except ALP obtained by liver function test were significantly higher in the study groups (p=0.014, 0.021, 0.014, and 0.009, respectively) compared with Group 1. In the evaluation of elevated transaminase levels, some diagnostic approaches, including the "De Ritis rate," have been determined. In 1957, Fernando De Ritis noted the importance of the ratio between serum AST and ALT levels. In the case of AST/ALT>1.5, intrahepatic cholestasis should be considered[8]. In this study, results were consistent with this rate. In Group 4, treated with 500 mg/kg *R. pimpinellifolia* L. fruit extract, AST, ALT, total bilirubin, and GGT levels were significantly reduced, and therefore, a therapeutic dose of 500 mg/kg could be more plausibly supposed to be optimal for preventing/reducing liver hepatocyte injury.

Considering antioxidant features, statistical significance could not be achieved in therapeutic groups. However, most of the oxidative stress parameters were highest in Group 5. This was attributed to dose-related toxication risk, suggesting that the extract may have a pro-oxidant effect at higher doses.

Myeloperoxidase (MPO) plays an important role in tissue damage in both acute and chronic inflammation. It acts as a key enzyme in the generation of reactive oxygen species promoting inflammation and oxidative stress[9]. In our series, MPO and 3-nitrotyrosine were high in liver homogenate in therapeutic groups, though it was not reflected in the serum. A recent study investigating MPO levels in non-alcoholic steatohepatitis patients showed consistent findings with our results. There was no difference in serum MPO levels despite prominent hepatic inflammation and MPO-expressing cell counts in liver biopsies[10]. However, we anticipated that serum effects may be observed in a later phase requiring a longer study period considering all other parameters as well.

Some studies reported that oxidative damage of proteins occurs in liver injury caused by CCl_4[11]. While oxidative damage to DNA, protein, or lipid is extremely harmful, proteins are more susceptible because they often act as catalysts inside cells. The non-enzymatic modifications of proteins through Amadori reactions in the early phase and AGEs can accumulate as protein modifications[12]. In our study groups, glycoxidation products, such as Amadori products and AGE, were found to be significantly lower in serum levels of treatment groups. However, liver homogenate analysis revealed significantly higher Amadori levels which were indicative of early damage, while there was no significant difference in terms of AGE representing the advanced stage degeneration. In this case, it is thought that *R. pimpinellifolia* L. extract might be successful in protecting against liver injury, especially in the early stages of mild-moderate injury situations.

Lipid peroxidation has an important role in human health. The reaction of oxygen with unsaturated lipids produces different types of oxidation products[13].

We focused on main products such as LOOH which is formed due to fatty acid oxidation during the early stages of lipid peroxidation and MDA which is one of the secondary products of lipid peroxidation. A significant decrease in LOOH was observed in the treatment groups. This significant change in early lipid peroxidation parameters such as LOOH and no change in parameters in the later phase of lipid peroxidation may contribute to the idea of plausible efficacy of *R. pimpinellifolia* L. in the early stages of the damage.

This study has limitations. Although a sample size calculation before beginning the study was performed, it was smaller compared with similar studies concerning limited laboratory space and financial constraints in addition to animal welfare. We anticipated that, because this herbal product is new, the findings of this study may offer a potentially positive influence on this patient population.

CONCLUSION

It is quite clear that phytotherapeutics can be very useful as a full or complementary therapy for many clinical conditions,

but it is necessary to provide more *in vivo* information[14]. These results showed for the first time that *R. pimpinellifolia* L. fruit extract enriched with flavonoids and polyphenolic compounds possesses significant *in vivo* anti-inflammatory activity manifested by combating particularly early-stage oxidative stress parameters involved in bile duct ligation-induced cholestatic liver injury. To determine the safety profile of *R. pimpinellifolia* L. fruit extract and to establish the "no observed adverse effect level" of the extract, adequately powered studies with larger sample size and with long periods of follow-up, including *in vitro* and *in vivo* toxicological assessments, should be performed.

INFORMED CONSENT STATEMENT

All data from this research, which was planned as an animal study, will be made available upon request. The authors state that the information may be published in the data sharing statement. Data include Excel and/or SPSS version of results obtained for statistical analysis, hematoxylin-eosin stained preparations, immunohistochemistry stained preparations, and any other information obtained.

AUTHORS' CONTRIBUTIONS

MKD: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. **PY:** Conceptualization, Investigation, Supervision, Writing – review & editing. **OC:** Data curation, Methodology, Resources, Validation. **KY:** Data curation, Formal Analysis, Methodology, Validation, Visualization. **MBYO:** Data curation. **ZGD:** Formal Analysis, Investigation, Software, Visualization, Writing – review & editing. **BC:** Funding acquisition, Resources, Supervision. **OBG:** Methodology, Supervision. **PA:** Resources, Supervision.

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ORIGINAL ARTICLE

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Effect of intensive training on immune system cells in elite female weightlifters

Mehmet Ali Karaselek[1*], Serkan Kuccukturk[2], Tugce Duran[3]

SUMMARY

OBJECTIVE: This study aimed to investigate the effects of intense weightlifting training on lymphocyte and natural killer cell subgroups, which are the major cells of the immune system, in elite female weightlifters.

METHODS: A total of 20 elite female weightlifters were evaluated using flow cytometry before training (pre-T), immediately after training (post-T), and after a 120-min rest period (rest-T).

RESULTS: Post-T and rest-T showed significant decreases in helper T (Th) and cytotoxic T compared with pre-T (p=0.045, p<0.001 and p=0.05, p<0.001, respectively). B and natural killer cells were higher in post-T and rest-T than in pre-T. The increase in B cells was significant in pre-T/rest-T (p<0.001) but not in pre-T/post-T (p=0.122). Intense training significantly increased natural killer cells in both post-T and rest-T (p<0.001). $CD56^{bright}$ and $CD56^{dim}$ natural killer cell subgroups were significantly lower in post-T and rest-T than in pre-T (p=0.005, p=0.006 and p<0.001, p=0.004, respectively).

CONCLUSION: This study shows that intense weightlifting alters peripheral lymphocyte and natural killer subgroup ratios, being the first investigation in this field.

KEYWORDS: CD4. CD8. CD19. NK.

INTRODUCTION

Weightlifting relies on strength, which is crucial for success[1]. Regular intense training enhances power generation[2]. Some studies show balanced exercise benefits immune cells[3-6]. Conversely, strenuous long-term exercise harms the immune system, increasing infection risk, especially in athletes[4-8].

Training affects lymphocytes, with lymphocytosis observed during and immediately after exercise, returning to pre-training levels within 24 h[3]. Some studies on weightlifting athletes reported decreased lymphocyte and leukocyte values post-training[9,10]. Lymphocytes include T and B cells, while natural killer (NK) cells are divided into $CD56^{bright}$ and $CD56^{dim}$ subsets. $CD56^{dim}$ cells are cytotoxic, and $CD56^{bright}$ cells secrete cytokines[11]. No study investigating peripheral lymphocyte and NK subgroups in male and female weightlifting athletes was found.

This study aimed to investigate the effect of 120-min weightlifting training (90–100% load) on CD4+, CD8+, CD19+ B, CD3-CD16+56+ NK cells, and subgroups ($CD56^{bright}$ and $CD56^{dim}$) in female weightlifters using flow cytometry.

METHODS

Participants

A total of 20 elite female weightlifters (≥18 years) who had been actively participating in national teams for the past 3 years were included. Exclusion criteria were <18 years of age, <3 years of sports experience, musculoskeletal issues, recent surgery/trauma, hematological/systemic disease, and medication affecting blood values. In addition, care was taken not to collect blood from the athletes during the menstrual period. For this purpose, the menstrual periods of the athletes were questioned and the blood required for the study was taken within 5–12 days, which is the earlier period in the menstrual cycle. The Institutional Review Board approved the study (2023/029; December 24, 2022), and written informed consent was obtained from participants.

Study design

In January 2023, 3 mL of K3 EDTA blood samples were collected three times: pre-training (pre-T; 60 min before), post-training (post-T; 10 min after), and rest period (rest-T; 120 min after) for analysis.

[1]Necmettin Erbakan University, Faculty of Medicine, Department of Internal Medicine – Konya, Turkey.
[2]Karamanoğlu Mehmetbey University, Faculty of Medicine, Department of Medical Biology – Karaman, Turkey.
[3]KTO Karatay University, Faculty of Medicine, Department of Medical Genetic – Konya, Turkey.
*Corresponding author: malikaraselek@gmail.com

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Complete blood count analysis

A complete blood count was performed with Cell-Dyn 1800 (Abbott Diagnostics, Abbott Park, IL, USA). White blood cell (WBC) (10^3/μL), neutrophil (10^3/μL), and lymphocyte (10^3/μL) counts were analyzed from each blood sample.

Flow cytometric peripheral lymphocyte and natural killer subgroup analysis

Peripheral lymphocyte and NK cell subgroup analysis involved gradient centrifugation using Ficoll-Hypaque for cell isolation. Surface staining was conducted with specific monoclonal antibodies (mAbs). For lymphocyte subsets, CD45, CD3, CD4, CD8, and CD19 mAbs were used, while, for NK and NK cell subsets, CD3, CD16, and CD56 mAbs were added. Following incubation and washing, cell count and analysis were performed using flow cytometry (BD Canto II) and the FACSDiva software. Absolute values were calculated using the [(WBCx1000)/% cell ratio] formula.

Training procedure

Athletes followed a 120-min training program comprising a 15-min warm-up (static flexibility, joint mobility, stretching, and balance exercises), a 90-min main training (3 sets of maximal repetitions at 90–100% load with auxiliary exercises for weightlifting movements), and a 15-min cool-down (static flexibility and stretching exercises).

Statistical analysis

Data normality was assessed with the Shapiro-Wilk test. Two-tailed tests were used with $p<0.05$ as the significance threshold. Mean and SEM were reported in the tables. One-way repeated-measures ANOVA was conducted to analyze the main effects across measurement points (pre-T, post-T, and rest-T), followed by Bonferroni correction for post-hoc comparisons. Statistical analyses were performed using the open-source jamovi statistical platform (Version 1.2.1.1) [The jamovi project 2021, Sydney, Australia, Jamovi. Retrieved from https://www.jamovi.org].

RESULTS

The study included 20 female elite weightlifters with a mean age of 18.47±1.61 years. Significant training-related changes were observed in WBC, lymphocyte, neutrophil, and peripheral lymphocyte/NK cell subgroups ($p<0.05$) (Table 1 and Figure 1).

Complete blood count analysis results

Pre-T/post-T and pre-T/rest-T comparisons showed significant increases in WBC, neutrophil, and lymphocyte counts. The elevation in WBC count was significant in pre-T/post-T and pre-T/rest-T ($p<0.001$), while the change between post-T/rest-T was not significant ($p=0.073$). Lymphocyte count significantly increased in all three comparisons ($p<0.001$; $p<0.001$; and $p=0.01$). Neutrophil count significantly changed in pre-T/post-T and pre-T/rest-T ($p=0.03$), but not in post-T/rest-T ($p=0.74$) (Tables 1 and 2).

Peripheral lymphocyte subgroup analysis results

Peripheral lymphocyte subgroup analysis compared changes in CD3+CD4+ Th, CD3+CD8+ CTLs, CD19+ B, and CD16+56+ NK cells during post-T and rest-T periods with pre-T. The cells significantly decreased in post-T and rest-T periods compared with basal values, with statistical significance in pre-T/post-T and pre-T/rest-T comparisons ($p=0.045$ and $p<0.001$, respectively). The change between post-T and rest-T was also statistically significant ($p<0.001$). CTLs decreased in post-T and rest-T, with non-significant reductions in pre-T/post-T and pre-T/rest-T ($p=1.0$ and $p=0.102$), but with a significant change between post-T and rest-T ($p<0.001$). B cells

Table 1. The results of the parameters measured before, after, and during the training period.

Parameters	Pre-T	Post-T	Rest-T	p-value
WBC (10^3/μL)	5.6±0.32	6.94±0.36	7.54±0.35	<0.001
Neutrophil (10^3/μL)	3.41±0.32	4.4±0.26	5.09±0.25	<0.001
Lymphocyte (10^3/μL)	1.5±0.11	1.92±0.12	1.81±0.08	0.003
CD3+CD4+ (10^3/μL)	2463.74±174.52	2339.04±200.92	2076.68±180.61	<0.001
CD3+CD8+ (10^3/μL)	1692.25±170.08	1654.31±113.89	1357.72±108.42	0.018
CD19+ (10^3/μL)	730±61.56	833.21±61.2	1913.16±102.43	<0.001
CD16+CD56+ (10^3/μL)	592.82±48.08	904.13±68.67	1225.02±77.32	<0.001
$CD56^{bright}$ (%)	1.60±0.39	0.18±0.02	0.22±0.02	<0.001
$CD56^{dim}$ (%)	2.84±0.46	0.34±0.06	1.07±0.24	<0.001

significantly increased in post-T and rest-T compared with pre-T, with significant increases in pre-T/post-T, pre-T/rest-T, and post-T/rest-T (p=0.012, p<0.001, and p<0.001, respectively) (Tables 1 and 2).

Natural killer cell subgroup analysis results

NK cells significantly increased due to training, showing statistical significance in all comparisons: pre-T/post-T, pre-T/rest-T, and post-T/rest-T (p<0.001, p<0.001, and p<0.001, respectively). $CD56^{bright}$ cell rates decreased in post-T compared with pre-T (p=0.005) and increased in rest-T compared with pre-T (p=0.006). $CD56^{dim}$ cell rates decreased in post-T compared with pre-T (p=0.005) and increased in rest-T compared with pre-T (p=0.004) (Tables 1 and 2).

DISCUSSION

Intense training affects weightlifters' immune system and its cells[12,13]. This study investigated changes in immune system cells during pre-T, post-T, and rest-T in weightlifting athletes using flow cytometry. Th and CTLs decreased in post-T and rest-T, while B and NK cells increased. Limited literature exists on peripheral lymphocyte subgroups in weightlifters, making our findings valuable for comparison with other sports studies.

The immune system plays a crucial role in protecting the body from microorganisms and maintaining its health. Exercise has been reported to have both positive and negative effects on the immune system, especially with intense and long-term exercise[14,15]. Exercise regulates the immune system by influencing leukocytes, which are the major immune cells[16]. Intense training generally

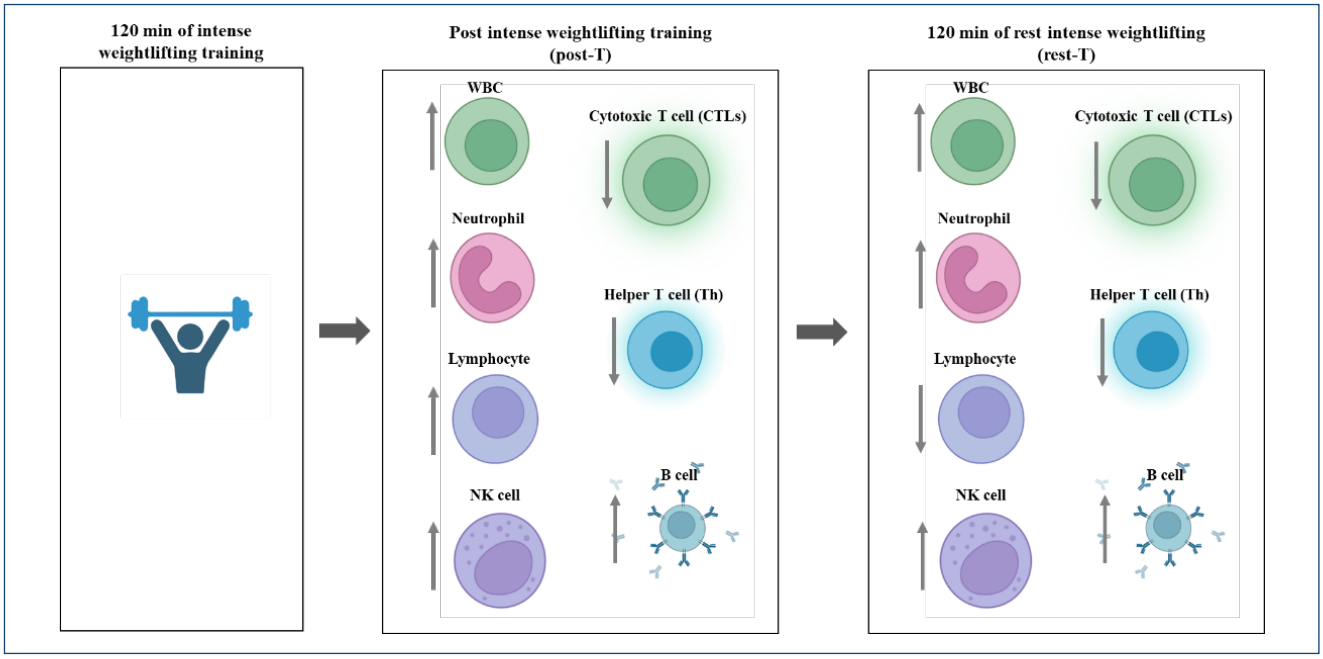

Figure 1. Change in peripheral lymphocyte subgroups due to training.

Table 2. Comparative statistical post-hoc analysis results of parameters.

Parameters	Pre-T/Post-T	Pre-T/Rest-T	Post-T/Rest-T
WBC ($10^3/\mu L$)	<0.001	<0.001	0.073
Neutrophil ($10^3/\mu L$)	0.001	<0.001	0.01
Lymphocyte ($10^3/\mu L$)	0.03	0.03	0.74
CD3+CD4+ ($10^3/\mu L$)	0.045	0.045	<0.001
CD3+CD8+ ($10^3/\mu L$)	1	0.102	<0.001
CD19+ ($10^3/\mu L$)	0.012	<0.001	<0.001
CD16+CD56+ ($10^3/\mu L$)	<0.001	<0.001	<0.001
$CD56^{bright}$	0.005	0.006	>0.05
$CD56^{dim}$	<0.001	0.004	0.008

leads to leukocytosis, but the response of leukocyte subgroups may vary[13,17,18]. In our study, WBC values significantly increased from pre-T ($5.6x10^3/\mu L$) to post-T ($6.94x10^3/\mu L$) and rest-T ($7.54x10^3/\mu L$), aligning with literature findings. Lymphocyte count typically rises during and immediately after exercise and then returns to pre-exercise levels within 30–60 min[13,17,18]. A recent meta-analysis also reported an immediate increase in total lymphocyte count after exercise, followed by regression within 1–2 h[19]. In our study, lymphocyte count significantly increased in post-T ($1.92\times10^3/\mu L$) and rest-T ($1.81\times10^3/\mu L$) compared with pre-T ($1.5\times10^3/\mu L$). However, the decrease in rest-T was not statistically significant compared with post-T. While post-training lymphocytosis aligns with the literature, the sustained elevation in the resting period after training may be specific to weightlifting, as other sports studies showed different patterns.

A meta-analysis on training-related peripheral lymphocyte subgroups indicated that Th cells returned to basal values within 1 h after exercise (p=0.74), CTL cells decreased (p=0.001), and NK cells increased above basal values (p=0.01)[19]. Studies in the literature consistently report decreased T cell proliferation during and after exercise. Trained male athletes showed significant reductions in mitogen-stimulated T cell proliferation following increased treadmill exercise[20]. Similar findings were observed in female athletes who underwent 2.5 h of treadmill running and cycling training[21]. In another study, comparing jogging at 80% VO_{2max} for 45 min with the same exercise at 50% VO_{2max}, lymphocyte proliferation decreased by 50 and 25%, respectively[21]. The changes in immune cells and recovery time after exercise are closely linked to exercise duration and intensity. In our study, Th and CTL cells decreased in the post-T period and did not return to pre-T levels during the 120-min rest-T period. Although our findings align relatively well with the literature, they contradict the data showing no return to baseline within the 120-min period. This discrepancy may be attributed to the specific sport type and intensity, highlighting the different responses of immune system cells to different sports training.

Senescent T cells theoretically undergo apoptosis, while naive T cells from the thymus enter the periphery, maintaining the peripheral lymphocyte count. Naive lymphocytes increase 1 h after exercise, and after 2 h, they transition to the periphery, restoring the lymphocyte count[22]. Exercise intensity is believed to increase apoptosis rates in T cell subgroups, leading to decreased cell numbers, which aligns with the findings of our study[23]. Furthermore, our study demonstrates that the intensity of weightlifting training induces more substantial decreases in T cell subgroups, with varying recovery times for these cells.

Unlike Th cells, B cells exhibit an increased response to exercise. Limited literature is available on weightlifting athletes. Turner et al. found that B cells nearly doubled immediately after exercise in healthy non-athletes but returned to baseline within 60 min[24]. In athletes performing at maximum capacity, B cell counts doubled post-training and tripled after resting[22]. The impact of training on B cells remains unclear in the literature. Despite varying data, the increase in B cells during post-T and rest-T periods aligns relatively well with the existing literature, suggesting that the sport type and training intensity may contribute to these differences, similar to Th cells.

The effects of exercise on NK cell subgroups are still not clear, with limited studies available. Our study revealed an overall increase in NK cell count but a decrease in $CD56^{bright}$ and $CD56^{dim}$ cells. Elite swimmers demonstrated an increase in $CD56^{bright}$ and a decrease in $CD56^{dim}$ NK subgroups[25]. NK cell responses to training vary across studies, with some reporting an increase and others a decrease[19,25]. Therefore, our study contributes to understanding the impact of intense exercise on NK cell subgroups, highlighting their dynamic nature.

The decrease in immune system cells in weightlifting female athletes poses an infection risk. Unlike some sports, T cells did not fully recover within 120 min after weightlifting training. Thus, ensuring an adequate rest period, post-training can minimize the athletes' susceptibility to potential infections.

CONCLUSION

Regular training is crucial for athletes' success, but inadequate rest can lead to significant changes in immune system cells. While studies on immune system cells exist for various sports, none specifically focuses on weightlifting athletes. This is the first study to examine the effects of weightlifting on peripheral lymphocyte and NK cell subgroups. It revealed that the decrease in cells due to intense weightlifting training did not fully recover within 120 min of rest. Long-term follow-up studies investigating cell recovery times could greatly contribute to mitigating infection risks for athletes. Hence, this study has the potential to inform future research in this field.

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AUTHORS' CONTRIBUTIONS

MAK: Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing. **SK:** Formal Analysis, Methodology, Validation, Visualization. **TD:** Methodology, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Antibiotic versus cranberry in the treatment of uncomplicated urinary infection: a randomized controlled trial

Oya Güven[1*], Samet Sayılan[2], Özlem Tataroğlu[3], Nihat Müjdat Hökenek[3], Dilek Vural Keleş[4]

SUMMARY

OBJECTIVE: This study was designed to determine the effect of cranberry extract used in patients with single urinary tract infections.

METHODS: Patients with simple-type urinary tract infections were divided into two groups. Treatment with fosfomycin or cranberry tablet was started. On days 1, 3, and 7 of the treatment, whether there was a decrease in the complaints was evaluated with a Likert-type scale. The recovery status of urinary tract infections and the well-being of patients were compared via antibiotic and cranberry groups.

RESULTS: After the treatment, the leukocyte levels of the cranberry users were at the same level as those of the other group, and the rate of well-being and the portion of patients that reported to be "very well" on days 3 and 7 in the cranberry group was significantly higher compared with the fosfomycin group ($p<0.05$).

CONCLUSION: Considering the results of this study, it was determined that the patient's complaints decreased from day 3 and their well-being increased with the use of cranberry only. Specifically, on day 7, the well-being of the cranberry group was higher than that of the fosfomycin group. For this reason, cranberry is a favorable alternative to antibiotics in uncomplicated and simple urinary tract infections.

KEYWORDS: Cranberry. Fosfomycin. Urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI), which is reported to be the second most common infection worldwide, is a condition that presents itself with alarming symptoms in women, and most of these women are treated with antibiotics[1,2]. Moreover, UTI may cause significant morbidity because of high recurrence rates and antibiotic resistance. In patients who frequently come down with UTI, this condition adversely affects gastrointestinal flora, liver, and kidney metabolism. Also, it causes a significant burden on the economies of countries.

The uropathogenic *Escherichia coli* (UPEC) pathogroup of *Escherichia coli*, which is classified into several pathogenicity groups according to particular virulence characteristics, is the most common causative agent of uncomplicated UTIs. *Staphylococcus saprophyticus*, a Gram-positive bacterium, is the second most common cause of uncomplicated UTI. Besides, *Klebsiella pneumonia* and *Proteus mirabilis* are other less common causes of UTIs. However, the Gram-positive bacteria *Enterococcus* and *Staphylococcus* have been more commonly detected as the causes of UTIs than studied previously. Due to these factors, UTIs will remain widespread[3].

Due to increased antibiotic resistance resulting from the widespread overuse and abuse of antibiotics such as beta-lactams, trimethoprim-sulfamethoxazole, and quinolones in many different countries around the world, for routine treatment of bacterial infections, including UTIs, it is observed that interest in non-antibiotic therapies for the management of these conditions is growing[4]. As a result, scientists are constantly searching for new strategies and therapeutic alternatives to antibiotics for the prophylaxis and treatment of UTIs. Cranberry, a fruit widely recommended for the prophylaxis of UTIs in traditional medicine, has emerged as a new alternative to antibiotics against UTIs and has become a new research subject in this field[5].

Cranberry fruit (*Vaccinium macrocarpon*) is a distinctive source of polyphenols such as flavonoids and phenolic acids, which have high antioxidant properties and are known to positively affect health[2]. It is available in cranberry, tablet/capsule, dried fruit, and cranberry juice/extract. Proanthocyanidin (PAC) with type A bindings or metabolites is considered the active ingredient in cranberries. It is stated that cranberry extract can be a potential alternative to antibiotics in treating acute UTIs

[1]Kırklareli University Medical School, Kırklareli Training and Research Hospital, Department of Emergency – Kırklareli, Turkey.
[2]Kırklareli University Medical School, Kırklareli Training and Research Hospital, Department of Internal Medicine – Kırklareli, Turkey.
[3]University of Health Sciences, Dr. Lutfi Kırdar City Hospital, Department of Emergency – İstanbul, Turkey.
[4]Kırklareli University, Faculty of Health Science, Department of Nursing – Kırklareli, Turkey.
*Corresponding author: ersinoya@yahoo.com
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without complication, particularly considering factors such as the reduction of the ability of *E. coli* by preventing it from adhering to the bladder uroepithelium[6].

There is still uncertainty about the effectiveness of non-antibiotic therapies for UTIs, and relevant studies on cranberries and other new combined formulations are going on. However, it is observed that most studies have evaluated the efficacy of cranberry extract in recurrent UTIs. Still, only a few studies evaluate its effectiveness in acute UTIs[7]. Therefore, in this study, we aimed to determine the effect of using cranberry extract in uncomplicated UTIs (that involve only the urinary tract and bladder, not kidneys).

METHODS

Ethics approval and consent to participate

This study was approved by the Local Ethics Committee and the Medicines and Medical Devices Agency (2021-514-212-7/E-E-85521274-000-1364973). All study participants gave written and verbal consent to the study. This study was carried out in accordance with the Declaration of Helsinki principles.

Study sample

This study was conducted as a prospective, randomized study. All male and female patients who presented to the Emergency Department (ED) and the Internal Medicine Outpatient Clinic between January 2022 and February 2022 were included in the study. The study was conducted with 170 patients (n=85 cranberry and n=85 fosfomycin).

This study included willing male patients and female patients who were not pregnant or suspected to have a pregnancy, who met the inclusion criteria of being aged 18 years or older, presenting to the ED and Internal Medicine outpatient clinic with complaints of dysuria, frequent urination, and nausea, having no known chronic kidney disease, and having only elevated leukocyte levels in the urine test results without pathology in blood tests.

Methods for data collection

Patients who accepted to participate in the study were divided into either the treatment group or the control group according to the order of arrival (1:1). Patients in Group 1 were given 7 cranberry tablets in one pack of cranberries "36 PAC available in 1 tablet (Ocean Cranberry, Orzax)" to be used once a day (QD). Patients in Group 2 were prescribed two sachets of fosfomycin every 3 days (TID). The study was completed with 170 patients, with 85 patients in each group. The researchers collected study data through in-person and telephone interviews. Each data collection method took about 10 min for the participants to complete.

Availability of data and materials

This study was retrospectively registered on clinicaltrials.gov with the trial number NCT05260554.

Follow-up of patients

The variables of age, gender, and admission symptoms were recorded for the patients included in the study, and treatment of their choice was initiated. The tests for leukocyte count, C-reactive protein (CRP), and kidney functions were suggested among patients with complaints increasing in severity during treatment. The patient was excluded from the study when pathology was identified and switched to the appropriate treatment. During admission, patients were asked to rate how serious their complaints were using the Likert-type verbal psychometric scale[8] (Table 1). Patients were called for a control examination (or queried on the phone) on days 3 and 7 of their treatment to question and record their well-being status. This study determined how quickly the patients' complaints recovered based on their treatment methods. The efficacy of the treatment was compared considering the leukocyte values in the results of control urine tests performed for the patients who completed the treatment. The results were evaluated based on the thesis that cranberry extract can be an excellent alternative to antibiotics in treating simple, uncomplicated urinary infections.

Statistical analysis

Study data were analyzed with SPSS 28.0. The mean, standard deviation, median, minimum, maximum, frequency, and ratio values were used for the descriptive statistics of the data. The chi-square analysis was used to examine the distributions between groups. The Kolmogorov-Smirnov test was used for measuring the distribution of variables. The Mann-Whitney U test was used for analyzing independent quantitative data. The Wilcoxon test was used for analyzing dependent quantitative data.

Table 1. Likert-type scale.

1 point	So bad
2 point	Bad
3 point	Not bad
4 point	Well
5 point	Very well

RESULTS

Of all patients in the study, 117 (68.8%) were females, 53 (31.2%) were males, and their average age was 53.4 (13.6) years (Table 2). In the fosfomycin group, the leukocyte count before treatment was significantly higher ($p<0.05$) than in the cranberry group. After treatment, the leukocyte counts of the subjects who took cranberry were identical to those of the other group (Table 2).

The complaint of nausea during treatment was observed not to change significantly in either group ($p>0.05$) (Table 2). The patients were asked whether their complaints were decreasing daily, and it was determined that, while the number of participants who gave 4 points to the question became higher in the group using fosfomycin, most of the participants using cranberry gave 5 points. In the cranberry group, the rate of participants reported to be "well" and "very well" on day 3 and the rate of those said to be "very well" on day 7 were significantly ($p<0.05$) higher compared with the fosfomycin group (Figure 1).

DISCUSSION

In this study, most patients with UTIs were postmenopausal women. The literature has reported that uterine muscles weaken, and vaginal Lactobacilli decreases in menopausal women due to reduced estrogen. Moreover, it has been reported that UTI pathogens, particularly Enterobacteriaceae, are responsible for colonization because of the increased pH levels. It is also

Table 2. Demographic characteristics of patients, types of treatment applied, laboratory results, and well-being.

		Fosfomycin		Cranberry		p
		Mean±SD n-%	Median	Mean±SD n-%	Median	
Age (years)		51.9±13.2	54.0	55.0±14.0	57.0	0.182[m]
Gender	F	59–69.4%		58–68.2%		0.868[X²]
	M	26–30.6%		27–31.8%		
Leukocyte						
Before treatment (BT)		19.6±6.8	18.0	16.5±5.1	16.0	**0.001**[m]
Post-treatment (PT)		8.6±14.8	2.0	7.4±13.9	2.0	0.558[m]
BT/PT		-11.0±14.9	-14.0	-9.1±14.5	-12.0	0.080[m]
Intragroup change p.		**0.000**[W]		**0.000**[W]		
Well-being						
Day 1	So bad	1–1.2%		1–1.2%		**0.045**[X²]
	Bad	24–28.2%		13–15.3%		
	Not bad	53–62.4%		57–67.1%		
	Well	7–8.2%		14–16.5%		
Day 3	So bad	1–1.2%		0–0.0%		**0.038**[X²]
	Bad	17–20.0%		9–10.6%		
	Not bad	20–23.5%		15–17.6%		
	Well	40–47.1%		46–54.1%		
	Very well	7–8.2%		15–17.6%		
Day 7	So bad	7–8.2%		1–1.2%		**0.003**[X²]
	Bad	11–12.9%		10–11.8%		
	Not bad	4–4.7%		10–11.8%		
	Well	45–52.9%		27–31.8%		
	Very well	18–21.2%		37–43.5%		
Nausea	No	39–45.9%		33–38.8%		0.352[X²]
	Yes	46–54.1%		52–61.2%		

[m]Mann-Whitney U test; [w]Wilcoxon test; [X²]chi-square test. Statistically significant values are indicated in bold.

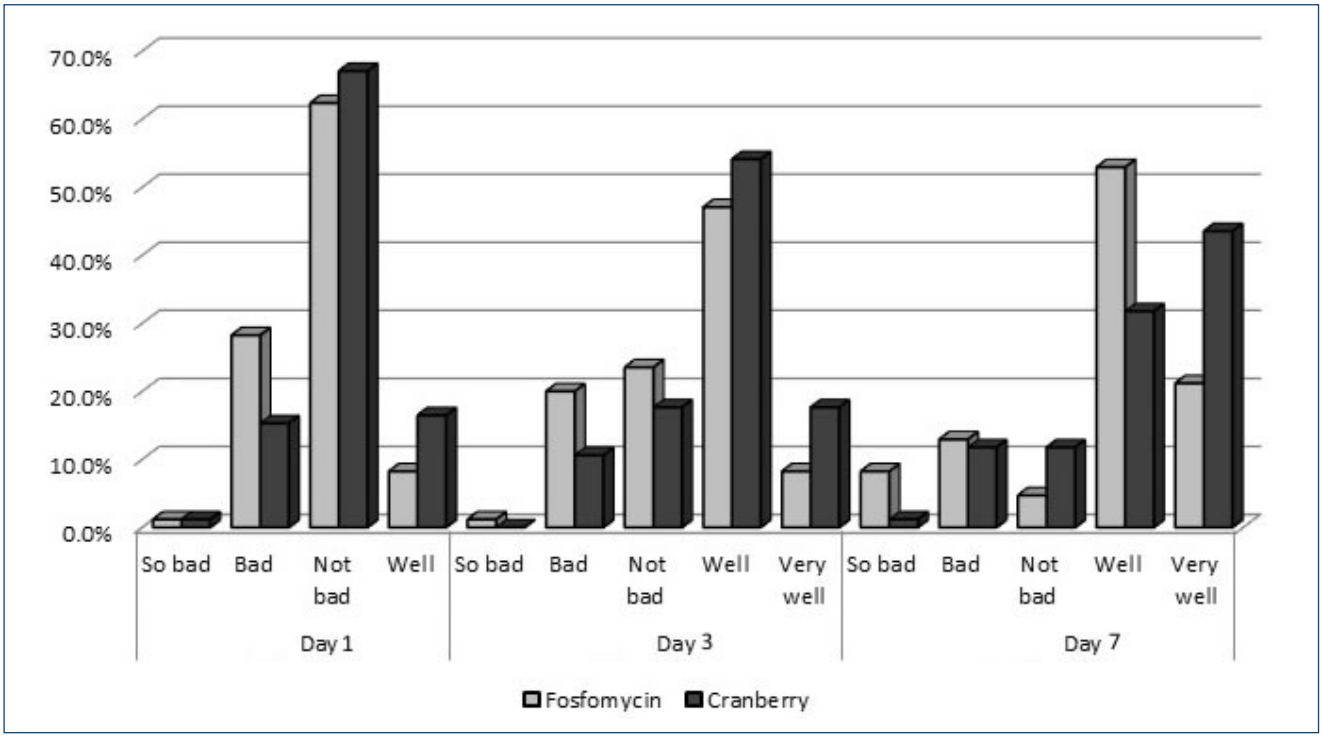

Figure 1. Distribution of well-being by days.

emphasized that UTIs may recur every 6–12 months after menopause[9]. Considering these facts, postmenopausal women have a higher risk of developing UTIs. For this reason, they form a higher portion of the study sample in this study.

The most significant characteristic of *E. coli* in UTI pathogenesis is its adherence to the host tissue with pili and fimbriae[10]. Type-1 fimbria and P-fimbria are the most important types of these structures. Following adhesion, uropathogenic are protected against micturition. Antibiotics and urinary antiseptics are often used in treatment. Cranberry fruit content includes water (88%), organic acids (including salicylate), fructose, vitamin C, flavonoids, *anthocyanidins,* catechins, and tri-terpenoids[11]. PAC in cranberry is considered the primary active ingredient and prevents Type-I and P-fimbriae adhesion of *E. coli* to the urogenital mucosa[12].

Cranberry is not an antimicrobial but can contribute to treatment as it indirectly inhibits bacterial adherence. It was demonstrated in a study by Sobota that, among the subjects who used diluted and concentrated cranberry juice, those who consumed the concentrate had less bacterial adhesion[13]. Faggian et al., observed that an increase in PAC polymers inhibited bacterial adhesion at the same rate[14]. This study used cranberry extract, which is high in PAC content (36 PAC=514 mg cranberry). When a comparison was made between the leukocyte levels in the urinalysis before and after the treatment, similar results were compared with the group using fosfomycin. This finding suggests that UTIs can be treated without using antibiotics.

In a study conducted to determine the effect of cranberry extract on antibiotic use in women with acute uncomplicated UTI, Gbinigie et al., concluded that it is possible to conduct randomized controlled studies with cranberry extract and that it has no side effects for patients[5].

In a study by Maki et al., the subjects were divided into two groups to receive cranberry juice and a placebo drink. As a result of the study, Maki et al., found that UTI episodes were reduced by 39% in subjects using cranberry juice[15]. Hess et al., compared cranberry tablets to placebo tablets in patients with neurogenic bladder[16]. There was a 60% decrease in UTIs in patients using cranberry tablets. Regardless of the form of cranberry used, successful results can be achieved with cranberry in treating UTIs.

Cranberries (*V. macrocarpon*) are generally cultivated in countries that have warm climates. As it is a cheap and easily accessible fruit, it can be thought that encouraging patients to consume cranberry will not be complicated by emphasizing its therapeutic effects. Furthermore, it has been demonstrated that cranberries protect against dental, gastrointestinal, and cancer and have antiviral activity[17-19].

In a study by Terris et al., it was determined that the risk of renal oxalate stones increases in patients using cranberry tablets

which has been attributed to vitamin C supplements in tablet form[20]. Additionally, patients with high levels of calcium oxalate had a familial history of kidney stones. Considering this result, it cannot be claimed that the cranberry tablet is good regarding the risk of developing kidney oxalate stones. However, there are several predisposing factors for this condition. Nevertheless, patients using cranberry extract should be monitored for renal stones.

CONCLUSION

This study determined that cranberry extract positively impacts the patient's well-being. Based on the data obtained, successful results can be achieved in treating UTIs using cranberries.

Limitations

The study population was limited to patients who presented to a single center between specific dates and were diagnosed with an acute UTI. Moreover, patients who had unresolved complaints were excluded from the study. Prolonging the treatment period could have been considered in that group of patients. However, further investigation was performed, and antibiotic therapy was started for those patients so as not to affect the study format. In addition, long-term follow-up of patients using cranberry could not be performed. It could not be followed whether side effects developed or not.

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AUTHORS' CONTRIBUTIONS

OG: Conceptualization, Data curation, Methodology, Software, Writing – original draft. **SS:** Conceptualization, Formal Analysis, Supervision, Writing – original draft. **ÖT:** Resources, Software, Supervision. **NMH:** Resources, Software, Supervision, Writing – review & editing. **DVK:** Validation, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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A retrospective study of pregnant patients with acute pancreatitis

Şehmus Ölmez[1], Bünyamin Sarıtaş[1*], Mehmet Suat Yalçın[2], Raziye Narin[3], Adnan Taş[1], Nevin Akçaer Öztürk[1], Mustafa Muslu[1], Haşim Nar[1], Ekrem Sapmaz[3], Banu Kara[1]

SUMMARY

OBJECTIVE: Acute pancreatitis is a rare disease in pregnant patients. Although it may have serious maternal and fetal consequences, morbidity and mortality rates have decreased recently due to appropriate and rapid treatment with earlier diagnosis. The aim of this study was to evaluate pregnant patients diagnosed with acute pancreatitis.

METHODS: The study included pregnant patients diagnosed with acute pancreatitis who were admitted to Adana City Training and Research Hospital in Adana, Turkey, between January 2014 and January 2022. Patients' files were screened. Patients' demographics, acute pancreatitis etiology, severity, complications, and applied treatment, as well as maternal and fetal outcomes were evaluated.

RESULTS: The study included 65 pregnant patients with acute pancreatitis. The mean age was 26.6±5 (19–41) years. Acute pancreatitis was observed in the third trimester. The most common cause of acute pancreatitis was gallstones, and its severity was often mild. Only two patients required endoscopic retrograde cholangiopancreatography, and the remaining patients were treated medically. Maternal and infant death developed in a patient with necrotizing acute pancreatitis secondary to hyperlipidemia.

CONCLUSION: The most common etiology of acute pancreatitis in pregnancy was gallstones. Acute pancreatitis occurred in the third trimester. Most of the patients had mild acute pancreatitis. Maternal and fetal complications were rare. We think that the reasons for the low mortality rate were mild disease severity and biliary etiology, and most patients were in the third trimester, as well as early diagnosis and no delay in the intervention.

KEYWORDS: Pancreatitis. Pregnancy. Prognosis.

INTRODUCTION

Acute pancreatitis during pregnancy (APDP) is a rare disease. The incidence of APDP varies and is 1/1000 to 3/10000 in pregnancies[1,2]. The prevalence of APDP is not different in nonpregnant patients[3]. The most common cause of APDP was gallstone, accounting for 36.4–70% of all cases[1,4,5]. The second-most common cause of acute pancreatitis (AP) is hyperlipidemia[6,7]. APDP caused by hyperlipidemia is associated with more maternal and fetal undesirable complications[4,6,7]. Recent studies have shown that the prognosis for APDP is not different from that of nonpregnant patients[8]. Due to improvements in both in diagnostic modalities and care in intensive care units and neonatal intensive care units, maternal and fetal mortality related to AP has decreased recently[1,4,6]. However, the development of AP in pregnant patients leads to serious stress in both patients and their relatives.

Acute pancreatitis is an important problem in gastroenterology clinical practice[9]. The most common causes of AP are gallstones, followed by hyperlipidemia and alcohol[10]. The clinical course of AP is classified as mild, moderate, and severe. In general, AP has a mild course, but it may have a severe course, leading to pancreatic necrosis, abscess, or organ dysfunctions even to death[9].

There are guidelines about the management of AP[9,11-13]. However, there is no guideline for the diagnosis and treatment of APDP. Since AP is rarely seen in pregnant patients and information about clinical follow-up and treatment is uncertain[3,4], it is very important to develop proper diagnostic algorithms and treatment strategies[5]. Besides, accompanying cholelithiasis and choledocholithiasis may affect the proper treatment choice and timing of treatment modalities such as endoscopic retrograde cholangiopancreatography (ERCP) or surgery. Patients must be followed up by a multidisciplinary approach, including gastroenterologists and obstetricians[5].

In this study, we aimed to evaluate patients with APDP, their treatment and prognosis, as well as maternal and fetal outcomes.

[1]University of Health Sciences, Adana City Training and Research Hospital, Department of Gastroenterology – Adana, Turkey.
[2]Muğla Training and Research Hospital, Department of Gastroenterology – Muğla, Turkey.
[3]University of Health Sciences, Adana City Training and Research Hospital, Department of Gynecology and Obstetrics – Adana, Turkey.
*Corresponding author: bunyamine@hotmail.com
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The study was conducted at the University of Health Sciences, Adana City Training and Research Hospital, Department of Gastroenterology, Dr. Mithat Özsan Bulvarı Kışla Mah. 4522 Sok. No. 1 Yüreğir/Adana, Turkey.

METHODS

Pregnant patients diagnosed with AP admitted to the gastroenterology department of Adana City Training and Research Hospital (Adana, Turkey) during the period of January 2014–January 2022 were included in the study. Patients' files and hospital computer databases were screened retrospectively. Patients' demographics, etiology of AP, and clinical and laboratory data were recorded. The duration of both hospital stay and intensive care stay, medical treatment records, maternal and fetal outcomes, and complications were recorded.

The diagnosis of AP and severity were made according to Atlanta criteria. The diagnosis of AP was made if two out of three criteria existed: 1. Abdominal pain (typical abdominal pain of AP is acute epigastric pain spreading to the back) 2. Above three times the upper limit of normal amylase and lipase levels. 3. Diagnostic imaging consistent with AP [computed tomography (CT), magnetic resonance imaging (MRI), or transabdominal ultrasonography (USG)]. Severity of AP was classified as mild if there were no signs of organ failure besides no local or systemic complications; moderate if there was transient organ failure (relieving in 48 h) and/or local or systemic complications without persistent organ failure (>48 h); or severe if there was persistent one or more organ failure according to revised Atlanta criteria[14]. Also, modified Ranson and modified Glasgow scores were also calculated to define the severity of AP[15,16].

Patients were categorized according to age. Patients' gestational age was determined according to the following: the first trimester was defined as weeks 1–13, the second trimester as weeks 14–27, and the third trimester as 28 weeks or longer gestational week.

Statistics

Statistical analysis was made with Statistical Package for the Social Sciences (SPSS) version 23 (IBM Inc.). Continuous variables are explained as mean±standard deviation (SD) (min–max), and categorical variables are given as frequency and percentage [n (%)].

RESULTS

A total of 65 patients were included in the study. The mean age was 26.42±5 (19–41) years. Of note, 36 (55.4%) patients were 19–25 years old, 16 (24.6%) patients were 26–30 years old, 9 patients were 31–35 years old, and 4 (6.2%) patients were 36–41 years old (Figure 1). The mean number of gravida was 2.75±2.1 (1–10), parity was 1.2857±1.58 (0–5), and abortus was 0.8276±1.41595 (0–5). Pancreatitis was observed in the third and second trimesters, respectively.

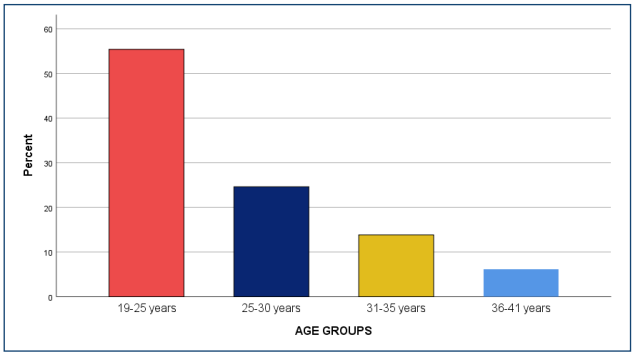

Figure 1. Distribution of patients according to age groups.

The most common etiologies of pancreatitis were biliary origin and hyperlipidemia. One patient had post-ERCP pancreatitis. Most of our patients [61 (93.8%)] had mild pancreatitis. The Ranson score and modified Glasgow score of our patients were 0.59±1.2 (0–6) and 0.53±0.76 (0–3), respectively. The mean duration of hospital stay was 4.3±2.5 (1–14) days for service and 0.9±3.9 (0–27) days for intensive care. Most of the patients were treated conservatively. Only two patients (3.1%) required ERCP. Only one patient with necrotizing pancreatitis secondary to hyperlipidemia required lipid apheresis, and maternal and infant death developed in that patient despite lipid apheresis. The patient was 30 years old, and had one parity with a healthy child and a history of AP. She died of AP related to hyperlipidemia during her second pregnancy. One patient had an early delivery at 37 weeks. Patients' demographic data, gravida and parity status, etiology, severity of pancreatitis, applied treatment modalities, and prognosis are summarized in Table 1. Patients' laboratory data are summarized in Table 2.

DISCUSSION

Although AP is a rare disease in pregnant women, the severity and etiology of pancreatitis should be determined to diagnose and treat these patients earlier, since it may have serious consequences in both the mother and the fetus[1,5,8]. Determining the exact trimester is also important to choose the correct treatment modality. Obstetricians should also evaluate the status of fetus at the beginning and when necessary. Determining the etiology of pancreatitis is very important since the treatment choice of ERCP, timing of cholecystectomy, or dietary modification affect the prevention of pancreatitis[6,9].

Pancreatitis in pregnant patients is diagnosed by using abdominal USG, abdominal CT, MRI, and endoscopic ultrasonography (EUS)[6]. In the selection of imaging method, the potential risks on the fetus should also be considered. Abdominal USG is the first diagnostic choice in the diagnosis and etiology of

APDP. It is noninvasive, cost-effective, and safe. But its diagnostic capacity is restricted, and it depends on the operator's experience, obesity, and intestinal gas. While the sensitivity of USG is good for cholelithiasis, it is poor for choledocholithiasis and pancreatitis[6,17]. Abdominal CT is commonly used in the diagnosis of AP, both in diagnosis and determining the severity of pancreatitis, but in pregnant patients its use is limited due to the potential risk of ionizing radiation and contrast agents on the fetus. So, it is not recommended in the APDP[6,17]. Magnetic resonance cholangiopancreatography (MRCP) is a very effective diagnostic modality in pregnant patients because it does not have ionizing radiation and contrast agents, and it is also very sensitive in diagnosing choledocholithiasis. EUS is also effective in diagnosing biliary stones and sludge, but it cannot be done in every case. It is performed under anesthesia. This method may be useful in patients for whom a high probability of choledocholithiasis is suspected but an abdominal USG or MRCP shows no biliary stone. It may be done prior to ERCP to prevent unnecessary ERCP procedures[17].

The etiology of APDP is similar in both pregnant and nonpregnant patients, as biliary stones are the most common etiology[1,2,7]. In normal pregnancy, some physiological changes occur in women. There is an increase in gallbladder volume, and the bile flow slows down. The most common contributing factors to these changes are increased estrogen and progesterone hormone levels[6]. In pregnancy, gallbladder stones and the frequency of biliary pancreatitis are increased[18]. Hyperlipidemia is another leading contributing factor to AP[7,8,19]. Other causes are drugs, trauma, pregnancy-induced hypertension, acute fatty liver disease of pregnancy, and genetic disorders. Idiopathic pancreatitis may also be observed[7,20]. Although alcoholic pancreatitis is frequently seen in the etiology of AP in nonpregnant patients, it is very rare in pregnant women. In some studies, it is not reported in the etiology of APDP[3,4,7,21]. Most studies reported that APDP was observed in the third trimester, and the most common cause was gallstone[6,19]. In our study, the most common etiologic factors were biliary stone disease

Table 1. Demographic and clinical information of patients.

Age (years) (mean±SD) (min–max)	26.42±5 (19–41)
Gravida	2.75±2.1 (1–10)
Parity	1.2857±1.58
Trimester of pregnancy	n (%)
°° First	11 (16.9%)
°° Second	15 (23.1%)
°° Third	39 (60%)
Etiology	n (%)
°° Biliary	50 (76.9%)
°° Hyperlipidemia	7 (10.8%)
°° Post-ERCP	1 (1.5%)
°° Idiopathic	7 (10.8%)
Severity of acute pancreatitis	n (%)
°° Mild	61 (93.8%)
°° Moderate	1 (1.5%)
°° Severe	3 (4.6%)
Ranson score	0.59±1.2 (0–6)
Modified Glasgow score	0.53±0.76 (0–3)
Duration of ICU stay (days)	0.9±3.9 (0–27)
Duration of inpatient follow-up (days)	4.3±2.5 (1–14)
Total duration of hospital stay (days)	5.1±4.7 (1–28)
Treatment	n (%)
°° Medical	63 (96.9%)
°° ERCP	2 (3.1%)
Prognosis	n (%)
°° Maternal mortality	1 (1.5%)
°° Fetal mortality	1 (1.5%)

ERCP: endoscopic retrograde cholangiopancreatography; SD: standard deviation; ICU: intensive care unit.

Table 2. Patients' laboratory data on admission.

	Mean±SD (min–max)
WBC (/μL)	12220±4053 (6900–27000)
Hb (g/dL)	11.3±1.4 (8.1–15.6)
CRP (mg/L)	13.2±23.9 (0.1–143)
Glucose (mg/dL)	106.3±26.5 (68–208)
AST (U/L)	92.8±126.6 (10–942)
ALT (U/L)	74.2±111.8 (3–497)
Alb (g/L)	35.4±4.4 (24.6–48.1)
T bil (mg/dL)	1.2±1.1 (0.2–5.2)
ALP (U/L)	146.9±87 (43–469)
GGT(U/L)	73.8±78.8 (6–424)
LDH (U/L)	297.8±172.8 (136–1175)
Ca (mg/dL)	8.8±0.6 (7.3–10.1)
BUN (mg/dL)	15.7±5.6 (4–32)
Cr (mg/dL)	0.43±0.12 (0.16–0.87)
NA (mmol/L)	135.6±4.4 (123–143)
K (mmol/L)	4.4±0.5 (3.1–6)

WBCs: white blood cells; Hb: hemoglobin; CRP: C-reactive protein; Alb: albumin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; T Bil: total bilirubin; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; LDH: lactic dehydrogenase; BUN: blood urea nitrogen; Cr: creatinine; Na: sodium; K: potassium.

(76.9%) and hyperlipidemia (10.8%). One patient had post-ERCP pancreatitis, for which ERCP was performed for choledocholithiasis. We have no cases related to alcohol. We have identified no etiologic factors in 10.8% of our patients. In our study, we observed AP in 39 (60%) patients in the third trimester, in 17 (26.2%) patients in the second trimester, and in 9 (13.6%) in the first trimester. In a meta-analysis including 823 patients, AP was observed in 64.9% of patients at the third trimester and decreased maternal and fetal mortality as gestational age increased. The highest maternal and fetal mortality was observed in the first trimester, and the lowest prevalence of AP was observed in the first trimester[4,8].

In a study conducted by Luo et al., which included 121 patients, it was reported that the most common etiology of APDP was biliary stones, followed by hyperlipidemia. Local complications were found to be higher in pancreatitis related to hyperlipidemia. Maternal and fetal mortality rates were correlated with the severity of AP, and they were 3.3% (4/121) and 11.6% (14/121), respectively[4]. In this study, high mortality rates may be related to a high number of pancreatitis cases due to hyperlipidemia. In a study by Tang et al., conducted on 54 patients, despite having no maternal mortality, fetal mortality was found in 11 patients (20.4%). In this study, the most common etiology of pancreatitis was hyperlipidemia and only one patient related to biliary stone had mortality[20]. In our study, only one patient died due to pancreatitis caused by hyperlipidemia. Fetal mortality occurred in the same patient. Only one patient had an early delivery at 37 weeks.

Although previous studies reported high maternal and fetal mortality rates with high undesirable outcomes, nowadays maternal and fetal mortality rates and undesired worse outcomes prevalence are lower. This may be due to patients' admission to intensive care units in the early period and developments in neonatal intensive care units[5,8].

Pancreatitis due to hyperlipidemia has worse outcomes than pancreatitis due to other etiologies. The exact mechanism of development of pancreatitis related to hyperlipidemia is unknown[20]. There are several theories of pancreatitis development and its more severe form in hyperlipidemia. High concentrations of chylomicron particles may lead to high blood flow resistance, leading to impairment in pancreatic microcirculation, and even Ischemia and necrosis may occur. Hydrolysis of triglycerides by pancreatic lipase may release free fatty acids, leading to excessive endothelial damage in acinar cells and pancreatic capillaries. At the same time, these free fatty acids may activate trypsinogen, which leads to severe pancreatitis and the activation of severe systemic inflammation. The severity of pancreatitis is correlated with the severity of hypertriglyceridemia[22]. Hyperlipidemia-associated APDP has worse fetal outcomes[20,23]. In pregnant patients, lipid-lowering drugs are contraindicated, and lipid apheresis or plasmapheresis may be done to lower triglyceride levels[23].

Studies reported that APDP was most observed in the third trimester, and high undesirable outcomes were observed in the first trimester in both fetus and mother[8]. In our study, pancreatitis was observed in the third, second, and first trimesters, respectively. Mortality and undesirable outcomes may be lower due to high number of patients in the third trimester[24].

In our study, most of our patients had mild pancreatitis according to Atlanta criteria, and Ranson and modified Glasgow scores were low. Most of our patients were treated conservatively. We observed lower maternal or fetal mortality in our patients. We have observed only one maternal mortality and one fetal mortality in the same patient. We thought that lower mortality rates may be related to a high number of mild pancreatitis and a high number of biliary pancreatitis, and most patients were in the third trimester. Besides, early admission of pregnant patients to the hospital and early beginning of proper treatment may also have an important role. Maternal death had occurred in a patient who had a second severe pancreatitis attack related to hyperlipidemia despite lipid apheresis.

Although developments in the treatment of AP in pregnancy and better outcomes have been achieved, AP causes serious stress in patients, their spouses, and relatives. Gastroenterologists and obstetricians must collaborate in the proper treatment and management of APDP for both mother and fetus.

In conclusion, we evaluated pregnant patients with AP, and the most common cause of AP was biliary. Most of the APDP was observed in the third trimester. Most patients had mild pancreatitis. Maternal and fetal complications were rare. We think that the reasons for the low mortality rate were mild disease severity and biliary etiology, and most patients were in the third trimester, as well as early diagnosis and no delay in the intervention.

AUTHORS' CONTRIBUTIONS

ŞÖ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft, Writing – review & editing. **BS**: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Sofware, Validation, Visualization, Writing – original draft, Writing – review & editing. **MSY**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Writing – original draft, Writing – review & editing. **RN**: Conceptualization, Data curation, Investigation,

Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **AT**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **NAÖ**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. **MM**: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **HN**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Sofware, Validation, Writing – original draft, Writing – review & editing. **ES**: Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. **BK**: Conceptualization, Investigation, Methodology, Project administration, Resources, Sofware, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Could a reduced hemoglobin, albumin, lymphocyte, and platelet (HALP) score predict autoimmune hepatitis and degree of liver fibrosis?

Muge Ustaoglu[1*], Gulali Aktas[2], Omer Kucukdemirci[1], Ibrahim Goren[1], Berk Bas[1]

SUMMARY

OBJECTIVE: Autoimmune hepatitis is a rare inflammatory disease of the liver that is characterized by elevated liver enzymes. The hemoglobin, albumin, lymphocyte, and platelet score, which is derived from hemoglobin, serum albumin, circulating lymphocyte count, and platelet count, is also associated with inflammatory conditions. The aim was to examine the hemoglobin, albumin, lymphocyte, and platelet score of patients with autoimmune hepatitis and to compare it to that of healthy individuals in this retrospective analysis.

METHODS: Subjects diagnosed with autoimmune hepatitis were enrolled in the study, and healthy individuals were enrolled as controls. Moreover, autoimmune hepatitis subjects were grouped into mild or moderate/advanced fibrosis. Furthermore, aspartate to platelet ratio index, Fibrosis-4, and hemoglobin, albumin, lymphocyte, and platelet scores of the autoimmune hepatitis patients and controls were compared. In addition, the hemoglobin, albumin, lymphocyte, and platelet score of the autoimmune hepatitis patients with mild fibrosis is compared to that of those with moderate/advanced fibrosis.

RESULTS: The mean hemoglobin, albumin, lymphocyte, and platelet score of the autoimmune hepatitis patients was 44.2±14.5 while this value was 76.8±15.5 in control subjects. The hemoglobin, albumin, lymphocyte, and platelet score was significantly reduced in autoimmune hepatitis patients than healthy controls ($p<0.001$). The hemoglobin, albumin, lymphocyte, and platelet score was significantly and negatively correlated with C-reactive protein, aspartate, alanine transaminase, gamma glutamyl transferase, aspartate to platelet ratio index, and Fibrosis-4 values. A hemoglobin, albumin, lymphocyte, and platelet score that was lower than 52.3 had 83% sensitivity and 73% specificity in predicting autoimmune hepatitis. The sensitivity and specificity of the hemoglobin, albumin, lymphocyte, and platelet score were higher than the Fibrosis-4 score in predicting moderate/advanced fibrosis in autoimmune hepatitis.

CONCLUSION: We suggest that the hemoglobin, albumin, lymphocyte, and platelet score be used as an additional noninvasive diagnostic tool for autoimmune hepatitis and to predict moderate/advanced liver fibrosis in patients with autoimmune hepatitis.

KEYWORDS: Autoimmune hepatitis. HALP score. Inflammation. APRI score. FIB-4 score. Fibrosis.

INTRODUCTION

Autoimmune hepatitis (AIH) is a rare liver condition characterized by elevated liver enzymes and positive specific antibodies in the serum of the affected subjects[1]. An increased burden of inflammation is a hallmark feature of autoimmune hepatitis[2,3] and other liver diseases[4].

Establishing the diagnosis of AIH is challenging. Therefore, novel markers are studied in this population to make a concise diagnosis and also to predict the outcome[5]. One of these novel markers is the hemoglobin, albumin, lymphocyte, and platelet (HALP) score. The association between inflammatory conditions, such as heart failure[6], intestinal obstruction[7], stroke[8], cancer[9], and HALP score, has been well established in the medical literature. However, there are no published works about the role of the HALP score in subjects with AIH.

We aimed to examine the HALP score of the patients with AIH and compare it to that of healthy individuals in this retrospective analysis.

METHODS

Design, setting, and population

Subjects diagnosed with AIH in outpatient gastroenterology clinics at Ondokuz Mayis University Hospital between August

[1]Ondokuz Mayis University, Faculty of Medicine, Department of Gastroenterology – Samsun, Turkey.
[2]Abant Izzet Baysal University Hospital, Department of Internal Medicine – Bolu, Turkey.
*Corresponding author: ustaoglu.md@gmail.com

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2020 and December 2022 were enrolled in the study after obtaining ethical approval from the local ethics committee (approval no. 2022/510). Control subjects were healthy individuals who presented to the internal medicine outpatient clinics of the institution for routine control. The exclusion criteria were as follows: younger than 18 years of age, active infectious or other inflammatory diseases, pregnancy, hematological conditions that alter platelet or lymphocyte counts, and any type of malignant condition. We also excluded subjects with advanced heart failure, chronic kidney disease, and chronic obstructive pulmonary disease.

Age, gender, and laboratory characteristics, such as serum biochemistry (urea, creatinine, aspartate transaminase [AST], alanine transaminase [ALT], albumin, C-reactive protein [CRP], alkaline phosphatase [ALP], and gamma glutamyl transferase [GGT]); autoimmune markers (anti-nuclear antibody [ANA], anti-mitochondrial antibody [AMA], anti-smooth muscle antibody [ASMA], and anti-liver kidney antibody 1 [anti-LKM1]); and hemogram parameters (white blood cell [WBC] count, neutrophil [neu] count, lymphocyte [lym] count, Hb, hematocrit [Htc], and platelet [PLT] count) were evaluated. Markers of common hepatitis viruses were recorded from patients' records. We also recorded liver biopsy findings, including histological activity index (HAI) score and fibrosis degree, in subjects with AIH.

We calculated the HALP score with the following formula: (Hb×serum albumin×lym)/PLT. AST to PLT ratio index (APRI) and Fibrosis-4 (FIB-4) scores were also calculated with the formulas AST/PLT and (age×AST)/(PLT×root square [ALT]), respectively. Data from the AIH and control groups were compared. We further grouped AIH patients into two: mild fibrosis group (fibrosis score: 0 or 1) and the moderate/advanced fibrosis group (fibrosis score: 2–6). Data from the mild and moderate/advanced fibrosis groups were also compared.

Statistical analyses

Statistical analyses were conducted with the SPSS software (SPSS 16 for Windows, IBM Co., Chicago, IL, USA). The homogeneity of the variables was analyzed with the Kolmogorov-Smirnov test. The comparison of the variables with a homogeneous distribution was done with an independent sample t-test. These variables were expressed as mean±standard deviation (SD). The variables without a homogeneous distribution were compared with the Mann-Whitney U test and expressed as medians (min–max). The comparison of categorical variables was done with a chi-square test and given in numbers and percentages. Correlation between study variables was conducted with Pearson's correlation test or Spearman's correlation test, where appropriate. The sensitivity and specificity of the HALP score in predicting autoimmune hepatitis were analyzed with the receiver operating characteristic (ROC) curve analysis test. When the p-value is lower than 5%, it is considered statistically significant.

RESULTS

The study population consisted of 204 subjects, with 112 in the AIH group and 92 in the control group. The mean ages of the AIH and control groups were 43.7±7.7 years and 41.1±9.8 years, respectively (p=0.17). Of note, 85 (76%) of the AIH group were women, while 64 (70%) of the control group consisted of female subjects. The genders of the groups were not statistically significant (p=0.31).

Serum creatinine (p=0.06), WBC (p=0.17), neu (p=0.054), and PLT (p=0.63) of the AIH and control groups were not statistically different. Serum albumin (p<0.001), Hb (p=0.001), and lym (p<0.001) levels of the AIH group were significantly lower than those of the controls. On the contrary, CRP (p<0.001), AST (p<0.001), ALT (p<0.001), GGT (p<0.001), and ALP (p<0.001) levels were significantly increased in AIH patients compared to the control subjects. Table 1 shows the data for the AIH and control groups.

The median APRI scores of the AIH and control patients were 1.88 (0.13–41.9) and 0.24 (0.11–0.53), respectively (p<0.001). The median FIB-4 scores of the AIH and control groups were 2.63 (0.19–20.6) and 0.72 (0.38–2.1), respectively (p<0.001). The mean HALP score of the patients with AIH was 44.2±14.5, while this value was 76.8±15.5 in control subjects. The HALP score was significantly lower in AIH patients than in healthy controls (p<0.001). The HALP score was significantly and negatively correlated with CRP (r=-0.15, p=0.04), AST (r=-0.33, p<0.001), ALT (r=-0.34, p<0.001), GGT (r=-0.19, p=0.008), APRI (r=-0.28, p<0.001), and FIB-4 (r=-0.26, p<0.001) values.

The ROC analyses revealed that an HALP score lower than 52.3 has 83% sensitivity and 73% specificity in predicting AIH (AUC: 0.88, p<0.001, 95%CI 0.84–0.93). Figure 1(a) shows the ROC curve of the HALP score in detecting AIH.

In addition, the sensitivity and specificity of AST (when higher than 33.1 U/L) in predicting AIH were 93 and 99%, respectively (AUC: 0.97, p<0.001, 95%CI 0.95–0.992). The sensitivity and specificity of ALT (when higher than 40.5 U/L) in predicting AIH were 88 and 99%, respectively (AUC: 0.93, p<0.001, 95%CI 0.89–0.97). The sensitivity and specificity of GGT (when higher than 58.9 U/L) in predicting AIH were 80 and 93%, respectively

Table 1. Data of the autoimmune hepatitis and control subjects.

		AIH	Control	p-value
Sex	Female [n, (%)]	85 (76)	64 (70)	0.31
	Male [n, (%)]	27 (24)	28 (30)	
		Mean±SD		
Age (years)		43.7±7.7	41.1±9.8	0.17
Albumin (g/dL)		4;04±0.64	4.45±0.29	<0.001
Hb (g/dL)		13±1.7	13.7±1.2	0.001
PLT (k/mm^3)		232±61	227±43	0.63
HALP score		44.2±14.5	76.8±15.5	<0.001
		Median (min–max)		
WBC (k/mm^3)		4.5 (2.4–21)	7 (4–10)	0.17
Neu (k/mm^3)		3.4 (1.4–15.7)	3.2 (1.2–6.9)	0.054
Lym (k/mm^3)		1.92 (0.6–8,8)	2.54 (1.1–4,8)	<0.001
Creatinine (mg/dL)		0.71 (0.37–1.58)	0.72 (0.67–0,92)	0.06
CRP (mg/L)		5.3 (0.3–162)	1.7 (0.2–3,6)	<0.001
AST (U/L)		124 (17–3785)	21 (13–43)	<0.001
ALT (U/L)		191 (10–3085)	28 (16–52)	<0.001
GGT (U/L)		114 (12–2861)	43 (33–61)	<0.001
ALP (U/L)		153 (23–1293)	67 (55–104)	<0.001
APRI		1.88 (0.13–41.9)	0.24 (0.11–0.53)	<0.001
FIB-4		2.63 (0.19–20.6)	0.72 (0.38–2.1)	<0.001

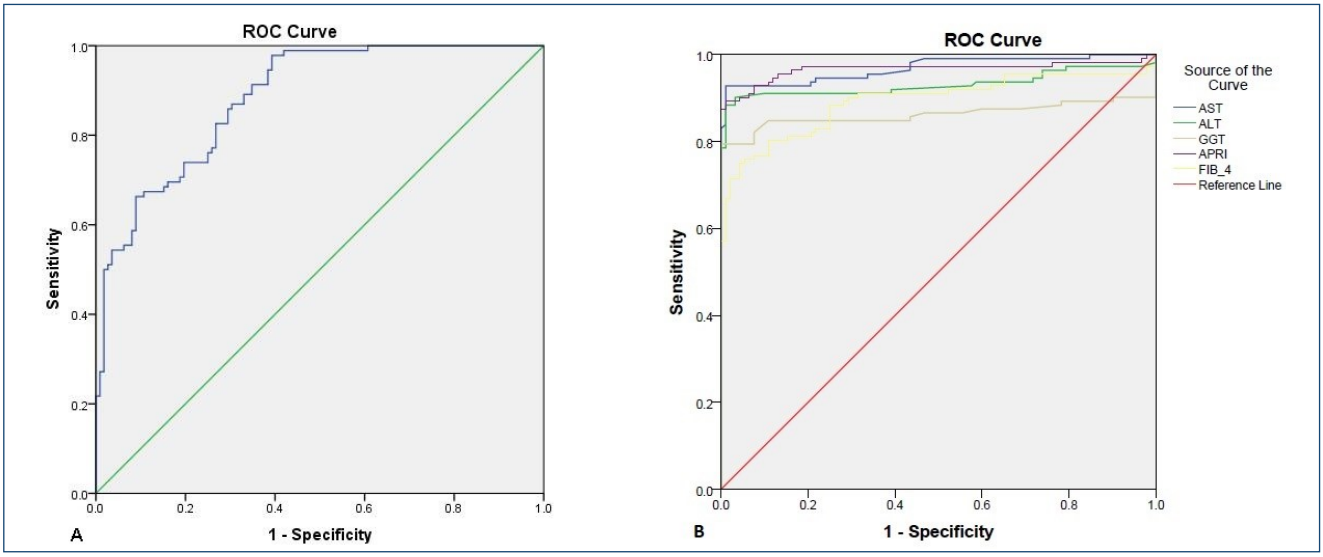

Figure 1. Receiver operating characteristic curve of hemoglobin, albumin, lymphocyte, and platelet score and other parameters in detecting autoimmune hepatitis.

(AUC: 0.86, p<0.001, 95%CI 0.80–0.92). The sensitivity and specificity of the APRI score (when higher than 0.35) in predicting AIH were 95 and 88%, respectively (AUC: 0.97, p<0.001, 95%CI 0.94–0.995). The sensitivity and specificity of the FIB-4 score (when higher than 0.99) in predicting AIH were 83 and 78%, respectively (AUC: 0.89, p<0.001, 95%CI 0.85–0.94). Figure 1(b) shows the ROC curves of these parameters for detecting AIH.

Subgroup analysis was performed in the AIH group, which was divided into mild and moderate/advanced fibrosis groups. The HALP score was significantly lower in AIH patients with moderate/advanced fibrosis (41.7±4.1) compared to those with mild fibrosis (48.1±4.5) (p=0.02). Moreover, the HALP score was negatively and significantly correlated with the fibrosis degree in the AIH subgroup (r=0.4, p=0.002). However, an HALP score lower than 43.4 had 66% sensitivity and 47% specificity in detecting moderate/advanced fibrosis in AIH patients (AUC: 0.6, p=0.009, 95%CI 0.49–0.70). The sensitivity and specificity of the HALP score were higher than the FIB-4 score but lower than the APRI score in predicting moderate/advanced fibrosis in patients with AIH. Figure 2 shows the ROC curves of HALP, APRI, and FIB-4 scores in predicting moderate/advanced fibrosis in AIH patients.

DISCUSSION

The present study showed that the HALP score could be used as an additional diagnostic tool in AIH since it is significantly reduced in these subjects compared to the controls. Another important outcome of our study is the significant and strong negative correlations between the HALP score and the levels of AST, ALT, GGT, APRI, and FIB-4 scores. Moreover, the HALP score was a useful predictor of advanced fibrosis in AIH subjects. Finally, the HALP score yielded considerably high sensitivity and specificity in establishing AIH diagnosis and predicting the degree of fibrosis in patients with AIH. A HALP score lower than 43.4 had 66% sensitivity and 47% specificity in detecting moderate/advanced fibrosis in AIH patients.

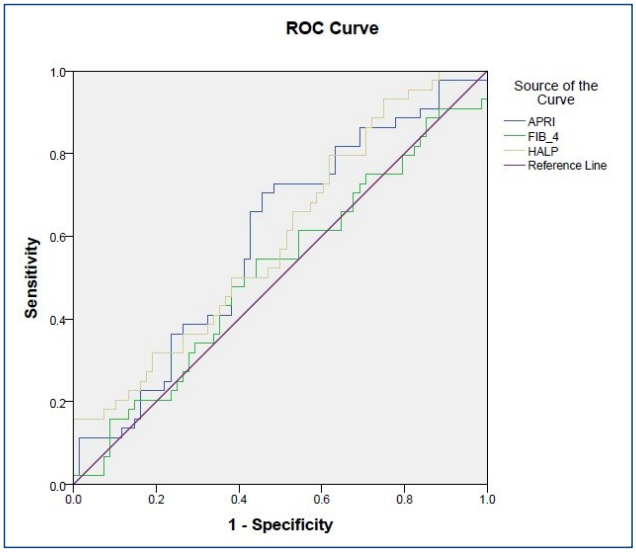

Figure 2. Receiver operating characteristic curves of the study variables in detecting moderate/advanced fibrosis.

We shall speculate here why the HALP score is reduced in AIH. Inflammation is a hallmark feature of autoimmune liver diseases, including AIH[10]. Both portal and lobular inflammation are reported based on histopathological examination of the liver biopsies of the patients with AIH[11]. Involvement of even T cells in the inflammatory cites in AIH was reported by Longhi et al., 2 years ago[12]. Many reports suggest this phenomenon. For instance, a Chinese study revealed alterations in the gut microbiome may have beneficial effects on inflammation in an experimental AIH model[13]. Inflammatory markers have been suggested to be predictors of diagnosis and prognosis in AIH population[14]. These data suggest that inflammation is a characteristic of AIH. Indeed, in another study, nimbolide, an inflammatory agent, is shown to reduce inflammation in the AIH model in mice[15]. Moreover, steroids are the treatment of choice in AIH, which modulates inflammation.

Various reports in the literature concluded that the HALP score could be useful for diagnosing and predicting the prognosis of several conditions characterized by overt or subtle inflammation. Liver cancer has been reported to be associated with a reduced HALP score[16]. A decreased HALP score is not only seen in liver cancer but also in other gastrointestinal cancers[17]. It is also associated with stroke[18], anti-neutrophil cytoplasmic antibody-associated vasculitis, and chronic obstructive pulmonary disease[19]. All of these conditions are associated with inflammation, as AIH is. Therefore, the association between AIH and the HALP score presented in this study is compatible with current literature.

The diagnosis of AIH is based on detectable autoantibodies, elevated to 1.1-folds of the upper limit of serum IgG, characteristic histological findings, and the absence of viral hepatitis[20]. We also established AIH based on this simplified criterion in the study population. Elevated aspartate and alanine transaminases, as well as GGT were common laboratory findings in patients with AIH. Similarly, we found elevated levels of these markers in patients with AIH compared to the control subjects, which is in line with literature data. Moreover, a significant and strong negative correlation between the HALP score and these enzymes was remarkable in the present study. Such a correlation between liver enzymes and red cell distribution width, a hemogram-based novel inflammatory predictor, has been proposed in a recent study[21]. Moreover, levels of CRP, a commonly used inflammatory marker, are correlated with liver enzymes and other markers of inflammation in AIH[22].

These data suggest that the correlation between liver enzymes and the HALP score presented in our study is consistent with literature data.

In one or two decades, APRI and FIB-4 scores have been introduced as prognostic markers of various liver diseases, from hepatitis to cirrhosis. Increased APRI and FIB-4 scores are suggested to be related to liver inflammation and fibrosis[23]. Moreover, elevated APRI and FIB-4 scores have also been reported in autoimmune liver diseases[3]. Similar to the literature findings, we reported increased APRI and FIB-4 scores in AIH patients compared to healthy controls. In addition, HALP score was also negatively correlated with these scores.

Several limitations are present in our study. Retrospective study design and a relatively small study population are two of these limitations. Another limitation can be stated as the single-centered nature of the study. However, to the best of our knowledge, this is the first study to find a significant association between the HALP score and AIH.

In conclusion, we suggest that the HALP score should be used by physicians as a noninvasive predictor in the diagnosis of disease in patients with suspected AIH and in predicting liver fibrosis in patients diagnosed with AIH.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICAL APPROVAL

The study was approved by the Ethics Committee of the Ondokuz Mayis University, Faculty of Medicine, and complied with the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

MU: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **GA:** Conceptualization, Formal Analysis, Investigation, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **OK:** Data curation, Methodology, Software, Validation, Writing – original draft. **IG:** Data curation, Investigation, Methodology, Resources, Writing – original draft. **BB:** Investigation, Methodology, Validation, Writing – original draft.

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ORIGINAL ARTICLE

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Comparison of choroidal thickness and choroidal vascular index in normotensive dippers and nondippers

Doğukan Cömerter[1*], Taha Baysal[1], Selami Doğan[2], Almina Erdem[2], Tufan Çınar[2]

SUMMARY

OBJECTIVE: The aim of this study was to evaluate the choroidal thickness and choroidal vascular index in normotensive individuals with dipping and nondipping patterns.

METHODS: Patients who applied to the cardiology clinic for routine checkups and underwent 24-h blood pressure monitoring were included in our study. They were divided into two groups based on their dipper status. The patients in whom systolic blood pressure decreased during the nocturnal time by 10% or more of the daily blood pressure were defined as dippers. On the contrary, patients whose nocturnal systolic blood pressure decreased by less than 10% were defined as nondippers. Choroidal thickness and choroidal vascular index were measured by spectral-domain optical coherence tomography. Central macular thickness, retinal nerve fiber layer, and ganglion cell layer (GCL) analyses were also recorded.

RESULTS: In total, 35 patients with dipper pattern and 34 patients with nondipper pattern were recruited. The mean subfoveal choroidal thickness was 349.72±90 μm in the dipper group and 358.54±132.5 μm in the nondipper group. The groups had no significant difference in choroidal thickness, central macular thickness, retinal nerve fiber layer, and ganglion cell layer analyses. However, the choroidal vascular index was statistically significantly lower in the nondipper group when compared to the dipper group (0.61±0.02 vs. 0.64±0.02; p<0.001). Also, the choroidal vascular index was negatively correlated with subfoveal choroidal thickness in the nondipper group (Spearman; r=-0.419; p=0.033).

CONCLUSION: Our study showed that the choroidal vascular index was significantly lower in nondippers than in dippers. Nondipper individuals may be affected by vascular dysregulation, leading to alterations in the choroidal circulation.

KEYWORDS: Optical coherence tomography. Disease, choroidal. Ambulatory blood pressure monitoring. Hypertension.

INTRODUCTION

Increased arterial blood pressure, also known as hypertension (HT), is a major risk factor for coronary heart disease, stroke, and renal failure[1]. Early and consistent detection of subtle microvascular changes in prehypertensive patients may provide prognostic information for cardiovascular risk stratification and disease progression[2]. Cardiovascular parameters, such as BP and heart rate (HR), change with the circadian rhythm throughout the day. Some studies show that both BP and HR in normotensive subjects decrease and remain relatively low throughout the night and then rise precisely in the early morning hours to reach a peak[3]. Patients with BP that does not decrease by 10% during night sleep compared to daytime are defined as "nondippers". Target organ injury, defined as clinical or laboratory finding of early hypertensive damage in any vascular organ, occurs more severely in nondipper hypertensive patients than in dipper patients[4]. Ambulatory blood pressure monitoring (ABPM) is commonly used to detect the dipper or nondipper pattern in BP readings[5]. The 24-h ABPM to identify dipping or nondipping pattern has become increasingly crucial for managing patients with pre-HT or essential HT[6].

The eye is a critical organ reflecting hypertensive microvascular effects and allows direct observation. Evaluation of visual parameters may provide a predictive and prognostic value in managing systemic complications secondary to HT, diabetes mellitus, and cardiovascular, cerebrovascular, and other systemic vascular diseases[7]. With the advances in imaging technology, SD-OCT provides visualization and measurement of the retinal layers and choroidal thickness (ChT). New OCT image modalities, including enhanced-depth imaging mode OCT (EDI-OCT) and optical coherence tomography-angiography (OCT-A), enable better visualization of the choroid and vascular plexuses in contrast to conventional techniques[8].

Several reports claim that changes in the microvascular structure of the choroid can be a sign of a systemic disease that affects blood vessels. Thus, the relationship between choroid and cardiovascular diseases (CVD) becomes an important clinical entity[9]. In the current literature, a prior study investigated the

[1]University of Health Sciences, Sultan Abdülhamid Han Training and Research Hospital, Department of Ophthalmology – İstanbul, Turkey.
[2]University of Health Sciences, Sultan Abdülhamid Han Training and Research Hospital, Department of Cardiology – İstanbul, Turkey.
*Corresponding author: dcomerter@hotmail.com
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ChT in hypertensive patients with nondipper and dipper patterns and found significant differences among these patients[10]. As early identification of vascular changes in normotensive patients with nondipper status may have an impact on the prognosis of such patients, this study aimed to compare ChT and CVI changes in normotensive dippers and nondippers.

METHODS

Study participants

This study was designed as a prospective, single-center study. The local ethics committee approved the study protocol and conducted it following the tenets of the Declaration of Helsinki (HNEAH-KAEK-2023/1106-4229). Informed consent was acquired from each subject before enrollment in the study.

The study group comprised 69 patients who applied to the cardiology clinic for the evaluation of blood pressure and were found to be normotensive based on 24-h AMBP monitoring. Patients with coexisting diseases such as diabetes mellitus, coronary artery disease, carotid artery stenosis, heart failure, renal failure, stroke, debilitating illness, hyperlipidemia, and dementia as well as patients with BMI>25 kg/m^2 were excluded from the study. In addition, patients with any ocular diseases (glaucoma, uveitis, and other retinal and neurodegenerative diseases), history of previous retinal treatment (laser photocoagulation and intravitreal injection), any intraocular surgery other than uneventful phacoemulsification, and media opacities that obscured the choroidal imaging were not included in the study.

Study protocol and procedure

Hypertension was defined as systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg, according to the 2018 European Society of Hypertension/European Society of Cardiology Guidelines for the management of arterial HT[11]. Based on the results of 24-h ABPM, patients who were found to be normotensive were classified into two groups according to their dipper status. Compared to daytime values, those whose night-time SBP decreased ≥10% were defined as dippers, and those whose SBP decreased <10% were defined as nondippers. Ophthalmic examination and SD-OCT imaging were performed by the same physician (D.C.) in a blinded manner. Ophthalmic examination included best-corrected visual acuity (BCVA) with Snellen chart, anterior segment evaluation with slit-lamp biomicroscopy, intraocular pressure (IOP), and funduscopic examination. All study participants had a BCVA of 20/20 and IOP lower than 21 mmHg. OCT images were obtained with the Spectralis OCT with eye-tracking dual-beam technology (Heidelberg Engineering GmbH, Heidelberg, Germany). One eye was randomly selected as the study eye. The fellow eye was studied if the randomly selected eye did not meet the inclusion criteria. CMT, RNFL, and GCL analyses were also recorded.

Choroidal thickness was measured manually using the caliper provided by EDI-OCT as the perpendicular distance between the hyperreflective outer border of the retinal pigment and the epithelial – Bruch's membrane – layer. The ChT was obtained at five different points in a horizontal scan line: the subfoveal, 500 µm and 1500 µm temporal to the fovea, and 500 µm and 1500 µm nasal to the fovea.

Choroidal vascular index was calculated using the ImageJ software (version 1.50a; NIH, Bethesda, MD, USA). The choroidal area (CA) was measured in a total of 3000 µm area (margin of 1500 µm nasal and temporal to the fovea center) horizontally and from the retina pigment epithelium (RPE) to the choroidoscleral border vertically. The edges of the CA were identified manually using the ImageJ ROI Manager. Binarization was executed using the Niblack auto-local threshold method. Dark pixel areas represent the vascular channels (luminal area, LA), and light pixel areas represent the stroma of the choroid (stromal area, SA) in the binarized image. CVI was calculated as the proportion of the LA to the total CA. Manual measurements were performed by the same physician (D.C) who is blinded to the groups. Measurements with a difference of more than 10% were excluded from the study.

Statistical analysis

Statistical analysis was analyzed by the SPSS program for Windows version 22. The Kolmogorov-Smirnov test was performed to determine whether continuous variables were distributed normally. The independent samples t-test and Mann-Whitney U-test were used to compare the quantitative data. For correlation analysis, the Spearman correlation analysis test was used. All values are given as means±standard deviations, and significance was considered $p \leq 0.05$.

RESULTS

This study included 35 patients (17 males and 18 females) with dipper patterns and 34 (14 males and 20 females) with nondipper patterns. The demographic and clinical characteristics of the subjects were similar at the baseline. There was no significant difference in the mean age, gender, daytime SBP, daytime DBP, night-time SBP, night-time DBP, and total BP values among the groups. As expected, the night-time SBP was higher in the nondipper group (115.85±11.1 mmHg vs.

112.64±9.9 mmHg). However, there was no statistically significant difference in BP parameters between the groups. BP parameters are shown in Table 1.

The mean subfoveal ChT was 349.72±90 µm in the dipper group and 358.54±132.5 µm in the nondipper group. There was no significant difference between the groups in ChT, CMT, RNFL, and GCL analyses. However, subfoveal ChT was positively correlated with daytime DBP (Spearman; r=0.464; p=0.017). OCT measurements are summarized in Table 2.

The CVI was statistically significantly lower in the nondipper group when compared to the dipper group. (0.61±0.02 vs. 0.64±0.02; p=0.0001). Additionally, the CVI was significantly negatively correlated with the subfoveal ChT in the nondipper group (Spearman; r=-0.419; p=0.033<0.05). A positive correlation was also found between CVI and night-time DBP when all participants were considered together (Spearman; r=0.301; p=0.032<0.05). Table 3 shows the relationship between subfoveal ChT, CVI, and BP values.

DISCUSSION

Choroid circulation has one of the highest rates of blood flow in the human body[12]. Choroid supplies oxygen and nutrients to the retinal layers between RPE and up to the inner nuclear layer[13]. Therefore, healthy choroidal vasculature is essential for normal functioning of the retina. Choroidal arteries have a unique structure in the choriocapillaris. Due to this morphology, high BP is transmitted directly to the choriocapillaris, and choroidal vessels are capable of blood flow autoregulation in response to changes in BP.

This study demonstrated the choroidal stromal and vascular changes in normotensive individuals with anomalous circadian BP patterns. Several previous studies have shown a better understanding of the vascularity of the choroid in both healthy and diseased eyes using SD-OCT[14-16]. However, previous studies mainly focused on ChT in cardiovascular diseases or eye disorders[17,18]. Tas et al.[10] reported that ChT in subfoveal and temporal locations were lower in the nondipper group, and they also found a negative correlation between night-time SBP and ChT. They focused on hypertensive patients with/without dipping patterns. In our study, we want to evaluate ChT and CVI in normotensive individuals with these patterns. We found that CVI values were statistically significantly lower in nondippers than in dippers. Moreover, a negative correlation was detected between CVI and subfoveal ChT in the nondipper group. We may explain these results with vasoconstriction and chronic

Table 1. Comparison of total, daytime, and night-time blood pressure in dipper and nondipper patients.

BP parameters (mmHg)	Dipper (n=35)		Nondipper (n=34)		p
	min–max	Mean±SD	min–max	Mean±SD	
Systolic BP, total	100–141	120±10.6	92–137	120.08±10.4	0.979
Diastolic BP, total	59–88	75.64±8.6	59–86	72.85±7.8	0.229
Systolic BP, daytime	100–136	121.08±10.4	96–140	120.92±10.1	0.957
Diastolic BP, daytime	60–90	76.8±9.3	61–94	75.35±8.7	0.566
Systolic BP, night-time	98–129	112.64±9.9	97–138	115.85±11.1	0.283
Diastolic BP, night-time	55–85	68.92±8.8	50–85	67.85±9.1	0.67

Table 2. Comparison of choroidal thickness, ganglion cell layer thickness, central macular thickness, and retinal nerve fiber layer thickness in dipper and nondipper patients.

Parameters	Dipper group (n=35)		Nondipper group (n=34)		p
	min–max	Mean±SD	min–max	Mean±SD	
Subfoveal ChT (µm)	203–566	349.72±90	131–610	358.54±132.5	0.992
ChT, nasal (500 µm)	140–561	345.6±104.8	96–581	362.46±133.8	0.62
ChT, nasal (1500 µm)	127–552	282.48±106.2	106–509	314±126.7	0.318
ChT, temporal (500 µm)	189–561	345.12±86.5	140–638	355.35±131.3	0.743
ChT, temporal (1500 µm)	200–552	315.08±89.9	107–561	328.73±111.4	0.559
GCL thickness (µm)	7–28	14.84±4.9	9–28	14.77±5.1	0.82
CMT (µm)	201–272	232.76±19.2	189–304	224.69±27	0.124
RNFL thickness (µm)	82–130	103.4±11.5	59–118	96.31±13.1	0.113

Table 3. Correlation between the choroidal vascular index, blood pressure, and subfoveal choroidal thickness.

CVI		Subfoveal ChT	Systolic BP, total	Diastolic BP, total	Systolic BP, daytime	Diastolic BP, daytime	Systolic BP, night-time	Diastolic BP, night-time
All participants (n=69)	r	-0.355	0.151	0.260	0.138	0.079	0.140	0.301
	p	0.011*	0.291	0.066	0.335	0.580	0.326	0.032*
Dipper group (n=35)	r	-0.304	0.295	0.199	0.242	0.061	0.368	0.430
	p	0.139	0.153	0.341	0.243	0.774	0.071	0.032*
Nondipper group (n=34)	r	-0.419	0.201	0.296	0.181	0.096	0.231	0.309
	p	0.033*	0.324	0.142	0.377	0.640	0.257	0.124

*indicates statistical significance ones.

Ischemia of vascular plexuses when anomalous BP patterns exist for a long time. Vasoconstriction in choroidal vessels, Ischemia, and RPE changes may play an essential role in the pathophysiology and progression of many choroidal and retinal diseases. Also, these findings suggest that CVI may be a more stable and reliable index for vascular status in CVD when compared to ChT.

Previous studies have described the thickening and thinning of the subfoveal choroid in the presence of CVD risk factors such as diabetes and hypertension[19-21]. The complex physiology of the choroidal vasculature, the impact of disease characteristics and medications, and even the retinal vasculature's status may complicate the ChT assessment in systemic diseases[22]. While Ahn et al.[23] found a significant correlation between the ChT and BP, other studies did not[24]. In our present study, we found no significant difference in BP parameters (daytime or night-time SBP and DBP). Also, no correlation was found between the ChT and BP values in all subjects; however, only subfoveal ChT was positively correlated with daytime DBP in the nondipper group. Gök et al.[20] reported that subfoveal ChT did not differ significantly between the dipper and nondipper hypertensive groups. Similarly, we found no significant difference in ChT measurements in normotensive individuals with dipping or nondipping patterns.

Lee et al., reported that the RNFL/GCL thickness ratios of the patients with chronic HT did not differ from the normal controls. In the other study, the RNFL thickness of hypertensive patients was thinner than healthy controls, which was most prominent in the superior and inferior quadrants[25]. In our present study, no significant differences in CMT, RNFL, and GCL were found between the groups. These inconsistent results may be due to undeveloped HT or the need for time to cause end-target organ damage.

Our study has some limitations, which must be considered when assessing our results:

1. Our sample size was small.
2. Since all of our study participants were Caucasians, our conclusion should be considered valid for this ethnicity only and cannot be generally applied to other ethnicities or genetic backgrounds.
3. The manual transmission of all identities of the Bruch's membrane and the inner scleral border, as Heidelberg SD-OCT equipment, did not automatically segment the choroid.

CONCLUSION

This is the first clinical study evaluating ChT and CVI in normotensive subjects with dipping and nondipping patterns. Our study showed that CVI was significantly lower in nondippers than in dippers. We found no difference in ChT between the groups. We discover that these anomalous BP patterns could affect choroidal changes in patients with CVD and should be considered when ChT and CVI are evaluated for chorioretinal diseases or other clinical studies. Larger, prospective studies will be needed to confirm our preliminary results, in particular, to clarify the role of nocturnal BP and nondipping patterns in normotensive or hypertensive subjects.

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INFORMED CONSENT

Informed consent was obtained from the patients included in the study.

ETHICAL APPROVAL

The study protocol was approved by the Haydarpasa Numune Training and Research Hospital Ethics Committee and conducted in accordance with the tenets of the Declaration of Helsinki (HNEAH-KAEK-2023/1106-4229).

AUTHORS' CONTRIBUTIONS

DC: Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Software, Supervision, Writing – original draft, Writing – review & editing. **TB**: Data curation, Formal Analysis. **SD**: Data curation, Formal Analysis. **AE**: Data curation, Formal Analysis. **TÇ**: Writing – review & editing.

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ORIGINAL ARTICLE

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Investigation of symptom management and functional state of women who underwent breast cancer surgery

Murat Can Mollaoğlu[1], Esra Başer Akın[2], Mukadder Mollaoğlu[2*], Kürşat Karadayı[3]

SUMMARY

OBJECTIVE: The aim of this study was to investigate the symptom management and the functional status of women who underwent surgery for breast cancer.

METHODS: This cross-sectional descriptive study was conducted in a university hospital surgical oncology clinic. This study was conducted on 80 patients who had undergone breast cancer surgery in the last 5 years in a surgical oncology clinic of a university hospital. Study data were collected using the patient identification form, Symptom-Management Self-Efficacy Scale Related to Chemotherapy in Breast Cancer, and Functional Living Index-Cancer. The data were analyzed with the SPSS program.

RESULTS: The mean total score of Symptom-Management Self-Efficacy Scale Related to Chemotherapy in Breast Cancer was found to be 157.28±36.86, and the mean total score of the Functional Living Index-Cancer was found to be 103.79±18.77. When the correlation between the Functional Living Index-Cancer and Symptom-Management Self-Efficacy Scale Related to Chemotherapy in Breast Cancer scales used in the study was examined, it was determined that there was a positive statistically significant correlation ($p<0.05$) between the subscale and scale total scores.

CONCLUSION: As a result of the study, it was determined that the self-efficacy and functional status of the patients were poor. Their functional status was also determined to be improved as the symptom self-efficacy levels increased.

KEYWORDS: Breast cancer. Disease management. Functional status. Symptom cluster. Health status. Mastectomy.

INTRODUCTION

Although surgical interventions are effective in treating cancer, treatment can often result in side effects that can affect the patient's function and quality of life (QoL). Even in the years after treatment, a functional decline is observed in patients[1]. Regardless of the type of surgical intervention, the functional status may be affected in patients in the following years due to pain, limitation of movement in the arms, and edema formation[2]. Functional status is a complex, multidimensional assessment of individuals' physical, psychological, and social well-being. Physical dimensions, work, and physical functionality include the ability to cope with psychological dimensions, self-acceptance, perceived health status, and adaptation to the disease[3].

Symptoms that develop in patients can negatively affect their QoL and functional status[4]. For this reason, it is thought that routine follow-ups will increase the QoL and functional independence of patients, as they provide the opportunity for symptom recognition and early intervention[1,4,5]. In addition, cancer reduces the individual's ability to cope with the disease, depending on the symptoms it causes in individuals. Thus, the physical, emotional, and social well-being of the patients is affected; they may feel powerless; and their self-efficacy perceptions may be negatively affected.

Self-efficacy is considered an effective component of well-being and successful symptom management. Self-efficacy, which plays an important role in facilitating health behavior and therefore improving health outcomes, is extremely important for cancer patients to cope with their symptoms and adapt to the process in their functional lives[5,6]. When the literature is examined, it has been observed that there are few studies examining self-efficacy and functional status in symptom management in patients after breast surgery[1,5-8]. In fact, no study that considers the two variables together has been found. The aim of this study was to examine symptom management and functional status of women who underwent breast cancer surgery and to evaluate the relationship between symptom management and functional status.

[1]Sivas Numune Hospital, Oncological Surgery Clinic – Sivas, Turkey.
[2]Sivas Cumhuriyet University, Faculty of Health Science, Department of Nursing – Sivas, Turkey.
[3]Sivas Cumhuriyet University, Oncological Surgery Clinic – Sivas, Turkey.
*Corresponding author: mollaoglumukadder@gmail.com
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METHODS

This cross-sectional study was conducted in a university hospital surgical oncology clinic. Patients who had undergone surgery for breast cancer in the surgical oncology department in the last 5 years were included in the study. In the surgical oncology clinic, patient records were examined, and patients who met the criteria were reached. Inclusion criteria for the study were as follows: having received at least two cures of chemotherapy or two cures of radiotherapy, volunteering to participate in the study, being 18 years of age or older, and not having a health problem that prevents communication.

In this study, when α=0.05, β=0.10, and 1-β=0.90 were calculated, 80 people were included in the study. The power of the test was obtained as p=0.90104 (PASS 2011 Home-Power Analysis and Sample Size)[9].

Research data were collected through the forms described below.

Patient information form

The demographic information about the patients and the data about the disease/treatment process were collected with a questionnaire form consisting of 21 questions prepared by using the literature.

Symptom Management Self-Efficacy Scale Related to Chemotherapy in Breast Cancer

In Liang et al.'s study, the SMSES-BC was developed to evaluate symptom management and self-efficacy in patients with breast cancer undergoing chemotherapy[10]. This scale consists of three subdimensions and 27 items: problem-solving skills (seven items), management of symptoms related to chemotherapy (15 items), and management of emotional interpersonal problems (five items). The score for the entire scale ranges from 0 to 270. The high score indicates that the individual's perceived self-efficacy in managing symptoms is high. Its validity and reliability in Turkish were studied by Semiz and Sağlam, and the Cronbach's α coefficient was found to be 0.90[11].

Functional Living Index-Cancer

Functional Living Index-Cancer was developed by Schipper et al., for use in cancer patients[12]. The scale consisting of 22 questions was prepared according to the 7-item Likert scale. The FLIC has five subtitles: Physical Functions, Psychological Functions, General Wellbeing (Cancer-related Challenges), Social Functions, and Gastrointestinal Symptoms. The lowest score that can be obtained from the scale is 22, and the highest score is 154. Low scores indicate poor functional status and QoL. The scale's validity and reliability in Turkey were studied by Bektas and Akdemir[13].

Statistical analysis

Descriptive statistics and correlational analyses were conducted. The SPSS version 18.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) program was used to evaluate the data obtained as a result of the research, and the number, percentage, mean, standard deviation, t-test, Mann-Whitney U test, and Kruskal-Wallis test were used.

Permission to conduct the study was obtained from the SCU Research Ethics Committee (decision no.: 2020-03/18). The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Participant characteristics and descriptive statistics are displayed in Table 1. Of the patients included in the study, 66.2% were between the ages of 45 and 65 years, 63.8% were married, 52.5% were primary school graduates, 36.2% had stage 2 cancer disease, and 67.5% had mastectomy (Table 1).

Symptom-Management Self-Efficacy Scale Related to Chemotherapy in Breast Cancer total score was evaluated as 157.28±36.86, and the FLIC total score was evaluated as 103.79±18.77.

As a result of comparing the sociodemographic characteristics of the patients with the SMSES-BC, the problem-solving subdimension and scale total scores of the patients aged 20–45 years were found to be statistically significantly higher (p<0.05). All subdimensions and total scores of those whose marital status was married were found to be high. The problem-solving, symptom management, and scale total score of the patients whose educational status was university and above were found to be statistically significantly higher (p<0.05). The scale total score of the patients who underwent breast-conserving surgery was found to be significantly higher (p<0.05). The management of symptoms subdimension and scale total scores were found to be statistically significantly higher (Table 1).

Table 2 shows the relationship between some sociodemographic characteristics of the patients and their functional life scale and subdimension means. According to the findings, the physical function subscale and scale total scores of the patients with an age range of 20–45 years were found to be statistically significantly higher. Physical, social function, general well-being subscale, and scale total score of the patients with university and higher education levels were found to be significantly higher than other education levels (p<0.05). The scale total score of the patients with stage 1 was evaluated to be higher than the patients in other stages. Physical function subdimension and scale total scores of patients who underwent breast-conserving

surgery were found to be statistically significantly higher than those who underwent mastectomy (Table 2).

When the correlation between the FLIC and SMSES-BC scales used in the study was examined, it was determined that there was a positive statistically significant correlation ($p<0.05$) between the subscale and scale total scores. As the level of self-efficacy in managing symptoms increases, functional life capacity and QoL also increase (Table 3).

DISCUSSION

In this study, which included women who had undergone breast cancer surgery, it was determined that symptom management and functional status were adversely affected. Similarly, studies on different populations of patients who have been operated on with breast cancer have determined that diagnosis and treatment negatively affect patients' self-efficacy and well-being[3,7]. Evaluation of patients' self-efficacy and functional status is one of the determining parameters of QoL and is an important parameter that clinical oncology attaches importance from past to present[1,6]. It has been reported that education and counseling on health promotion strategies can improve patients' self-efficacy and general well-being[4,6,8]. Increased self-efficacy not only helps patients effectively manage psychosocial variables associated with QoL but can also contribute to better compliance, positive treatment outcomes, and more informed treatment options.

In this study, the relationship of some sociodemographic and clinical characteristics of women with breast cancer with symptom management self-efficacy and functional status was also examined. Accordingly, symptom management self-efficacy was determined by age, marital status, educational status, and type of surgical intervention, whereas functional status was related to age, educational status, stage of the disease, and type of surgical intervention.

Self-efficacy and general well-being of women aged 20–45 years, the youngest group of the study, was found to be

Table 1. Comparison of sociodemographic characteristics and Symptom-Management Self-Efficacy Scale Related to Chemotherapy in Breast Cancer total and subscales.

Characteristics		n (%)	Problem-solving skills	Managing symptoms	Managing emotional interpersonal problems	Total
			X±S.D	X±S.D	X±S.D	X±S.D
Age (years)	20–45	10 (12.5%)	48.30±10.83	92.60±21.66	27.80±6.25	168.70±31.93
	45–65	53 (66.2%)	41.69±13.29	95.20±21.55	24.32±7.40	161.23±38.29
	65 and above	17 (21.2%)	31.17±10.87	83.29±17.49	23.76±5.19	138.24±29.17
	Statistical test		KW=12.68; p=0.002	KW=5.03; p=0.081	KW=2.39; p=0.303	KW=6.24; p=0.044
Marital status	Married	51 (63.8%)	42.96±12.40	96.11±21.97	26.17±6.79	165.25±36.83
	Single	29 (36.2%)	35.58±14.13	85.72±17.90	21.93±6.31	143.24±33.05
	Statistical test		t=2.42; p=0.017	t=-2.16; p=0.033	t=2.75; p=.007	t=2.66; p=0.009
Education	Illiterate	22 (27.5%)	28.09±9.07	78.36±17.80	21.72±5.36	128.18±24.82
	Primary school	42 (52.5%)	44.30±11.25	98.97±19.09	25.85±7.64	169.14±34.94
	High school	9 (11.2%)	42.44±13.38	89.88±24.78	24.11±6.43	156.44±37.33
	University	7 (8.8%)	51.71±13.07	99.71±18.35	27.14±4.59	178.57±30.26
	Statistical test		KW=27.70; p=0.001	KW=15.54; p=0.001	KW=6.42; p=0.093	KW: 21.71; p=0.001
Cancer stage	Stage 1	27 (33.8%)	43.62±11.64	97.03±13.74	27.11±7.04	167.78±28.35
	Stage 2	29 (36.2%)	40.20±13.88	91.72±26.17	23.96±6.63	155.90±43.71
	Stage 3	24 (30%)	36.62±14.35	87.83±20.78	22.66±6.45	147.12±34.54
	Statistical test		KW=3.16; p=0.206	KW=3.13; p=0.206	KW=3.84; p=0.147	KW=5.11; p=0.078
Type of surgery	Breast-conserving surgery	26 (32.5%)	43.69±12.21	96.19±13.90	27.11±6.91	167.00±29.45
	Mastectomy	54 (67.5%)	38.64±13.81	90.50±23.67	23.44±6.62	152.59±39.34
	Statistical test		U=560.50; p=0.146	U=576.50; p=0.197	U=531.50; p=0.79	U=511.00; p=0.050

X: mean; S.D: standard deviation; KW: Kruskal-Wallis; t: t-test; U: Mann-Whitney U test.

Table 2. Comparison of sociodemographic characteristics and Functional Life Scale-Cancer total and subdimension mean scores.

Characteristics		Physical functions	Psychological functions	General well-being	Social functions	Gastrointestinal symptoms	Total
		X±S.D	X±S.D	X±S.D	X±S.D	X±S.D	X±S.D
Age (years)	20–45	45.60±6.44	32.30±3.12	15.30±3.74	11.70±1.94	10.50±2.54	115.40±13.19
	45–65	39.01±10.42	29.52±5.46	13.83±4.14	10.62±2.60	10.90±2.29	103.91±19.76
	65 and above	34.47±8.66	29.23±4.71	12.70±3.63	10.88±2.11	9.29±3.01	96.58±15.25
	Statistical test	KW=8.05; p=0.018	KW=2.24; p=0.326	KW=3.59; p=0.166	KW=1.70; p=0.427	KW=3.77; p=0.152	KW=7.36; p=0.025
Marital status	Married	40.09±9.34	30.62±4.66	13.92±3.92	11.05±2.07	10.49±2.62	106.20±16.12
	Single	36.72±11.07	28.37±5.61	13.51±4.21	10.37±2.95	10.55±2.44	99.55±22.37
	Statistical test	t=−1.45; p=0.151	t=1.92; p=0.058	t=0.431 p=.668	t=1.20; p=0.232	t=−0.103; p=.918	t=1.53; p=0.129
Education	Illiterate	32.31±8.49	27.63±4.86	12.31±3.25	10.13±1.90	10.27±2.83	92.68±15.39
	Primary school	40.90±9.90	30.52±5.26	14.26±4.20	10.90±2.70	10.45±2.58	107.05±19.22
	High school	39.66±7.03	30.22±4.81	12.66±4.09	10.11±1.96	10.88±2.26	103.56±14.24
	University	46.28±9.69	31.85±3.89	16.85±3.07	13.28±0.75	11.14±1.95	119.43±14.22
	Statistical test	KW=14.65; p=0.002	KW=5.81; p=0.121	KW=8.98; p=0.029	KW=15.63; p=0.001	KW=0.573; p=0.903	KW: 15.86; p=0.001
Cancer stage	Stage 1	42.00±8.52	31.44±4.22	14.74±3.51	11.40±1.86	10.40±2.34	110.00±13.77±13.77
	Stage 2	38.79±10.43	29.27±5.07	14.34±3.68	10.86±2.50	10.44±3.00	103.72±20.11
	Stage 3	35.45±10.47	28.62±5.76	12.00±4.47	10.08±2.79	10.70±2.35	96.87±20.19
	Statistical test	KW=5.67; p=0.059	KW=4.03; p=0.133	KW=5.60; p=0.061	KW=23.25; p=0.197	KW=0.204; p=0.903	KW=6.89; p=0.032
Type of surgery	Breast-conserving surgery	42.88±8.00	31.26±4.40	14.84±3.39	11.46±1.74	10.61±2.24	118.08±13.44
	Mastectomy	36.94±10.44	29.11±5.31	13.25±4.21	10.50±2.66	10.46±2.69	100.28±20.03
	Statistical test	U=460.50; p=0.013	U=539.00; p=0.093	U=555.00; p=0.130	U=562.50; p=0.147	U=695.0; p=0.942	U=441.50; p=0.007

KW: Kruskal-Wallis, t: t-test, U: Mann-Whitney U test.

Table 3. The relationship between Symptom-Management Self-Efficacy Scale Related to Chemotherapy in Breast Cancer and Functional Living Index-Cancer.

FLIC / SMSES-BC		Physical functions	Psychological functions	General well-being	Social functions	Gastrointestinal symptoms	Total
Problem-solving skills	r	0.685**	0.447**	0.642**	0.323**	0.205	0.685**
	p	0.001	0.001	0.001	0.003	0.068	0.001
Managing symptoms	r	0.612**	0.384**	0.536**	0.371**	0.222*	0.626**
	p	0.001	0.001	0.001	0.001	0.048	0.001
Managing emotional interpersonal problems	r	0.613**	0.446**	0.560**	0.143	0.143	0.608**
	p	0.001	0.001	0.001	0.206	0.204	0.000
Total	r	0.708**	0.466**	0.645**	0.357**	0.228*	0.722**
	p	0.001	0.001	0.001	0.001	0.042	0.000

FLIC: Functional Living Index-Cancer; SMSES-BC: Symptom-Management Self-Efficacy Scale–Breast Cancer related to chemotherapy. ** and * indicate statistically significant values: *p<0.01, **p<0.05.

higher than other age groups in this study. Studies have reported that functional status regresses in advancing ages, depending on other increases in self-care activities, and therefore self-efficacy perception is negatively affected[8,14]. This finding is remarkable for monitoring and rehabilitating the functional status of elderly women who have undergone breast surgery. Thus, it can be ensured that older women get help earlier.

It was found that individuals whose marital status was married had higher symptom management self-efficacy levels in the study. Marriage, spouses providing support, and helping each other seem to be effective in managing symptoms, problem-solving, and emotional problems. In addition, this result can be explained by the strong social support of individuals[14]. When the literature was reviewed, it was determined that similar results were obtained in the studies[5,7].

Early diagnosis of cancer is effective in preventing metastasis, increasing the quality of life, and prolonging life expectancy. Surgical interventions determined according to the stage of cancer also have different effects on survival. A recent retrospective cohort study clearly demonstrated the negative impact of advanced cancer on survival outcomes[15]. In another randomized controlled study, it was determined that breast magnetic resonance imaging, due to its high sensitivity in detecting invasive neoplasms, detects cancer at an early stage and has a role in prolonging life[16]. As determined in this study and in a similar study[5], the functional status of survivors of early-stage breast cancer is better than those of advanced-stage breast cancer. For this reason, to increase the life expectancy and functional status of breast cancer patients, awareness of current diagnosis and treatment methods should be high, and these factors should be evaluated together with well-being.

In this study, FLIC and SMSES-BC scores were higher in patients who underwent breast-conserving surgery as a surgical intervention. In a study evaluating the QoL in women who underwent breast-conserving surgery and mastectomy, it was found that women who underwent mastectomy were more functionally affected[17]. One of the recent systematic reviews and meta-analysis, studies has brought to the attention of breast reconstruction surgeons that autologous fat grafting as a surgical intervention is a safe procedure in breast cancer and its role in improving both the survival and functional status of patients[18]. In other studies, it was found that the self-efficacy levels of women who had breast-conserving surgery were better[4,19]. This result is thought to be related to the effects of body image and functional capacity in women with mastectomy.

Studies have shown that individuals with high self-efficacy are more effective in managing the disease process and its treatments[4-8,19]. Similarly, in this study, as self-efficacy in symptom management increases, well-being in functional life increases. Therefore, it should be noted that high self-efficacy in patient care plays an important role in symptom management and improves functional well-being.

CONCLUSION AND RECOMMENDATIONS

Symptom management self-efficacy and functional status of women undergoing breast surgery were adversely affected in our study. In the study, symptom management self-efficacy was found to be high in young, highly educated, married women who underwent conservative breast surgery. On the contrary, the functional life status was found to be low in those who were elderly and who had low educational status, advanced disease, and undergone mastectomy. A positive and significant correlation was found between symptom management self-efficacy and functional status. Health professionals should contribute to increasing the knowledge and skills of patients on symptom management by providing education, counseling, and life coaching to patients with problem-solving interventions. Arrangements that increase self-efficacy and functional capacity with a multidisciplinary approach should be included in routine patient care.

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AUTHORS' CONTRIBUTIONS

MCM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **EBA:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MM**: Conceptualization, Methodology, Project administration, Supervision, Validation. **KK**: Conceptualization, Methodology, Project administration, Supervision, Validation.

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ORIGINAL ARTICLE

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Religion and sociodemographic characteristics at baseline of the Brazilian Longitudinal Study of Adult Health study

Ana Carolina Varella[1*], Itamar Souza Santos[1,2], Marcos Rafael Nogueira Cavalcante[1], Isabela Martins Benseñor[1,2], Paulo Andrade Lotufo[1,2]

SUMMARY

OBJECTIVE: The aim of this study was to investigate whether sex, age, race, income, education, and marital status are associated with having a religion in a sample of Brazilian men and women.

METHODS: Data were obtained from 15,098 participants of the Brazilian Longitudinal Study of Adult Health, a longitudinal study that ultimately aims to investigate long-term outcomes of chronic diseases. The sociodemographic characteristics and data on religion status were self-reported during interviews conducted by trained personnel. All study procedures followed standard and validated protocols.

RESULTS: There was a strong association between being a woman and having a religion (adjusted OR=2.12, 95%CI 1.95–2.31) when compared to men. Regarding age, those with 45–54 years were more likely to have a religion (adjusted OR=1.14, 95%CI 1.03–1.27). Blacks and Browns were more religious (adjusted OR=1.31, 95%CI 1.15–1.49, and OR=1.22, 95%CI 1.10–1.34, respectively) compared to Whites. Those with high income and education were less likely to state having a religion (adjusted OR=0.78, 95%CI 0.70–0.87, and adjusted OR=0.50, 95%CI 0.43–0.59, respectively). Those who did not have a stable conjugal union were found to be less religious (adjusted OR=0.82, 95%CI 0.75–0.89). Stratifying the analysis according to income showed that higher education was inversely associated with religion on both strata: lower and higher annual earnings.

CONCLUSION: This study suggests that education is one of the most important socioeconomic characteristics to consider when studying religion. Race, sex, income, and marital status are also important factors; however, there was not a clear association between religion and age.

KEYWORDS: Faith. Education. Religion. Epidemiology.

INTRODUCTION

Religious involvement has been studied over the past decades, and researchers have attempted to identify the determinants of such an engagement[1]. Sociodemographic characteristics are considered as important determinants of religious beliefs[2]. Sex, age, race, income, education, and marital status have a crucial role on how one perceives their surroundings and builds their personal beliefs[3]. Evidence suggests that these factors might lead people to either more traditional or liberal meaning systems. People with more traditional meaning systems would tend to be more religious[4].

According to the 2010 National Census, 92% of Brazilians stated having a religion (National Census, 2010)[5]. Even though the terminology "religion" does not reflect the religiosity or spirituality of an individual[6], it still reflects some extent of the personal belief. In the past few decades, Brazil has suffered a religious transition, when people change their inherited beliefs and circulate among various religious affiliations, sometimes returning to the primary one[7]. This religious transition is probably associated with sociodemographic and economic changes as well[7].

There are not many studies focusing on the relationship between sociodemographic characteristics and religion in the Brazilian population, and they often describe these characteristics by religious affiliation. Studying the characteristics of religious people helps elucidate how sociodemographic variables influence people's opinions and behavior toward religion[3].

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a cohort study including men and women from six different states in Brazil, with a socioeconomic gradient that grants good diversity to the sample allowing such investigation. The purpose of this study is to investigate whether sociodemographic characteristics are associated with religious status using baseline data.

[1]Universidade de São Paulo, Center for Clinical and Epidemiological Research – São Paulo (SP), Brazil.
[2]Universidade de São Paulo, Department of Internal Medicine – São Paulo (SP), Brazil.
*Corresponding author: acvarella@gmail.com
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METHODS

Study design and sample

Data were obtained from the baseline evaluation (August 2008 to December 2010) of ELSA-Brasil. It is a multicenter study located on six different cities of Brazil (Belo Horizonte, Porto Alegre, Rio de Janeiro, São Paulo, and Salvador e Vitória). At baseline, 15,105 civil servants between 35 and 74 years of age from institutions located in each of these cities were enrolled to participate. Exclusion criteria were as follows: intention to leave the institution at any time soon, severe cognitive or communication impairment, current or recent pregnancy (<4 months prior to the first interview), and if retired, reside outside of a study center's metropolitan area. ELSA-Brasil aims to study the incidence and associated risk factors of cardiovascular diseases and diabetes. The assessment consisted of interviews and clinical examinations carried out under strict quality control by trained personnel. The questionnaire covered a wide range of health-related topics, such as sociodemographic factors, cardiovascular risk factors, lifestyle, morbidity, cognitive function, medication use, mental health, and others[8,9]. More information about ELSA-Brasil can be found elsewhere[10-12].

From the original 15,105 participants, we excluded missing values for the religion variable (7); therefore, the final overall sample was 15,098.

Variables

Participants who responded yes to the question "Nowadays, do you have a religion?" were considered having a religion. Age was categorized as 35–44, 45–54, 55–64, and 65–74 years. Race was self-reported according to the Brazilian Census classification in the following categories: Black, Brown, White, Asian, and Indigenous. Marital status was dichotomized into without conjugal union (single, divorced, and widowed) or with conjugal union (married and others). Annual income was dichotomized using a cutoff value of US$ 20,000/year (at a rate of BRL 2.00=US$ 1.00), and education was categorized as less than high school, high school, and college.

Statistical analyses

All statistical analyses were conducted with SPSS Statistics for Windows, version 24. The categorical variables were described using the chi-squared test and presented as absolute numbers and proportions. Logistic regression models were used to analyze the association between having a religion (dependent variable) and the sociodemographic variables of interest (sex, age, race, education, income, and marital status). Unadjusted and adjusted models were analyzed. All variables with a p<0.20 in Table 1 were included in the process. The final model included age, sex, education, income, race, and marital status as independent variables.

As the results analyzing men and women separately were mostly similar, we do not present analyses stratified by sex. The significance level was set at 0.05.

RESULTS

Table 1 shows that about 77% of participants stated having a religion. Among them, 58.2% were women, 40.1% were between 45 and 54 years of age, 17.5% were of Black race, 52.1% had up to high school of education, 44.7% had lower income, and 69% had conjugal union.

Results from the logistic regression models (Table 2) showed sex, education, income, race, and marital status to be

Table 1. Sample characteristics by religion status.

	Having a religion		
	No 3506 (23.2)	Yes 11592 (76.8)	p-value
Age, years			
35–44	841 (24.0)	2497 (21.5)	0.001
45–54	1287 (36.7)	4649 (40.1)	
55–64	1006 (28.7)	3226 (27.8)	
65–74	372 (10.6)	1220 (10.5)	
Sex			
Men	2038 (58.1)	4846 (41.8)	<0.0001
Women	1468 (41.9)	6746 (58.2)	
Race			
White	2089 (60.8)	5697 (49.6)	<0.0001
Brown	832 (24.2))	3370 (29.4)	
Black	385 (11.2)	2012 (17.5)	
Asian	100 (2.9)	273 (2.4)	
Indigenous	32 (0.9)	125 (1.1)	
Education			
Less than high school	300 (8.6)	1622 (14.0)	<0.0001
High school	815 (23.2)	4418 (38.1)	
College	2391 (68.2)	5552 (47.9)	
Income, US$/year			
≤20,000	1002 (28.7)	5160 (44.7)	<0.0001
>20,000	2489 (71.3)	6380 (55.3)	
Marital status			
With conjugal union	2473 (70.6)	7999 (69.0)	0.081
Without conjugal union	1032 (29.4)	3593 (31.0)	

independently associated with religion. Women were found to be more religious than men (adjusted OR=2.12, 95%CI 1.95–2.31). People in the age group of 45–54 years were found to be more religious compared to those with the age group of 35–44 years. There was no difference among the other age strata. Education was inversely associated with religion, especially for those who completed college or more (adjusted OR=0.50, 95%CI 0.43–0.59). Income was inversely associated with having a religion (adjusted OR=0.78, 95%CI 0.70–0.87) as well. Brown and Black people were found to be more religious when compared to White people (adjusted OR=1.22, 95%CI 1.10–1.34; OR=1.31, 95%CI 1.15–1.49, respectively). After adjustment for all variables, people without a conjugal union were found to be less religious (adjusted OR=0.82, 95%CI 0.75–0.89).

Further analysis (Supplementary Table 1) stratified by income and adjusted for sex showed that those with higher education were more likely to not have a religion (adjusted OR=0.33, 95%CI 0.24–0.46), while people of Black and Brown races were more likely to have it (adjusted OR=1.64, 95%CI 1.34–1.99, and adjusted OR=1.26, 95%CI 1.11–1.43, respectively), among those with higher income. For those with lower income, there were differences in religion status only for those with higher educational level (OR=0.76, 95%CI 0.61–0.94).

DISCUSSION

This study showed an important inverse association between higher education and religion. A sensitivity analysis stratified by income showed higher education to be inversely associated with religion even for those with lower income. Women were more religious than men. Individuals of self-reported Black or Brown races were more frequently religious when compared

Table 2. Logistic regression models for the association between religion and sociodemographic variables.

	Unadjusted		Adjusted*	
	OR	95%CI	OR	95%CI
Sex				
Men	Reference		Reference	
Women[a]	1.93	1.79–2.09	2.12	1.95–2.31
Age, years				
35–44	Reference		Reference	
45–54	1.22	1.10–1.34	1.14	1.03–1.27
55–64	1.08	0.97–1.20	1.08	0.96–1.21
65–74[b]	1.11	0.96–1.27	1.23	1.06–1.43
Race				
White	Reference		Reference	
Brown[a]	1.49	1.36–1.63	1.22	1.10–1.34
Black[a]	1.92	1.70–2.16	1.31	1.15–1.49
Asian	1.00	0.79–1.27	0.91	0.72–1.16
Indigenous	1.43	0.97–2.12	1.07	0.71–1.59
Income, US$/year				
≤20,000	Reference		Reference	
>20,000[a]	0.51	0.47–0.55	0.78	0.70–0.87
Education				
Less than high school	Reference		Reference	
High school and some college	1.00	0.87–1.16	0.97	0.83–1.13
College or more[a]	0.43	0.38–0.49	0.50	0.43–0.59
Marital status				
With conjugal union	Reference		Reference	
Without conjugal union[a]	1.08	0.99–1.17	0.82	0.75–0.89

*Adjusted for age, sex, race, education, income, and marital status. [a]p<0.0001. [b]p<0.05.

to White individuals. Income was inversely associated with having a religion, and people with a conjugal union seemed to be more religious than those without one. Age was not clearly associated with religion.

We found women to be more religious than men, and this result remained significant after adjustment. Sex has been generally associated with religion, and women have been reported to be more religious than men[13,14]. Data from a survey conducted by the Committee on the Social and Psychological Factors Affecting Fertility in Indianapolis (US) reported greater interest in religion and religious practices among women when compared to men[15]. Our findings extend these results to a more diverse sample in terms of race. Additionally, in a cross-sectional study using data from the Sexual Behavior of the Brazilian Population and HIV/AIDS Perceptions Study in Brazil, Almeida & Monteiro[7] showed that a higher proportion of men considered themselves as not having a religion compared to women.

Age has been pointed out as important for religious engagement[16]. Analyzing the report by Almeida & Monteiro[7], we see that people under 25 and over 41 years of age were the most religious groups, showing an unclear yet discussible pattern of association. Younger people might still carry their parents' beliefs, followed by a life period when individuals experience other religious expressions. Possibly, in older ages, individuals decide to return to the primary belief or stay with the new one. A different unclear pattern was found in our study. We found that people between 45 and 54 years of age were more religious when compared to the youngest. Surprisingly, older age strata were not associated with having a religion in this sample. Religious dynamics in young adults cannot be fully explored with ELSA-Brasil data, as participants in our cohort aged at least 35 years at baseline. However, this study's results reinforce the idea that age is not a reliable predictor for religious status[1]. Age and religion relationship might be associated with religious affiliation and family beliefs[17].

This study also showed that having a conjugal union was positively associated with religious status after multivariate adjustment. The association between marital status and religion has been controversial. A study by Mormons reported no effect of marital status on religious behaviors[2]. However, Fisher[18] showed that marital status influences the nature of people's social relationships, and Cornwall[2] also showed that it could influence the social relations within a religious group. These latter findings could help to explain the nature of our results. It is suggested that marital status itself would not lead people to be more religious but could influence how they find groups to engage, such as more traditional environments that tend to value religious principles[4].

Another sociodemographic characteristic known to play an important role in religious beliefs is race[19,20]. We found Black and Brown people to be more religious than White people, and among all the three, people of Black race were the most religious group. Studies suggest a historical explanation for these findings, Black people would lean toward faith and get involved in religious groups in order to have comfort and help from a local community, once they historically have weaker political and societal support when compared to White people[17,21]. We found no difference in Asian and Indigenous people, which could be explained by the smaller sample size of those two races.

Our results showed a strong inverse association between education and having a religion, that is, the higher the educational level (college or more), the lower the frequency of people identifying themselves with a religion. Brazil is primarily a Christian country (Catholics and Protestants)[7], and these religious denominations are usually associated with lower educational levels. This present study showed that higher income was also associated with a lower frequency of people stating to have a religion. Some studies suggest that people in higher educational and socioeconomic levels tend to incorporate beliefs that shift toward the intellectual meaning instead of relying on faith[1,4].

Higher education usually means higher income, which is concentrated primarily among people of White race in Brazil[22]. Attempting to capture the possible relationship among those three characteristics and their influence on religion, we conducted a sensitivity analysis among participants with lower and higher incomes. Among those with higher income, the Black and Brown races were positively associated with religion, while higher educational level was inversely associated with having a religion. For those with lower income, the differences in religion status were seen only for those with higher educational level, and this could mean a strong association between religion and education independent of income.

Some limitations of this study include the fact that all variables were self-reported which could introduce some bias. Also, ELSA-Brasil was not meant to investigate religious involvement. Therefore, the variables included in the baseline assessment did not reflect the spirituality of the individual, but they still reproduce some extent of the personal belief, as some authors mentioned that there is still an overlap between the terms "religion" and "spirituality" for many people[23].

CONCLUSION

Among all sociodemographic factors studied, education seemed to have greater influence on religious status, followed by race, income, sex, and marital status. A clear trend of association with age was not observed.

ETHICAL APPROVAL

All procedures in this study were in accordance with the ethical standards of the 1975 Helsinki Declaration updated in 2013. The study was approved at all six centers by each Institutional Review Board, and all participants signed a written informed consent.

AUTHORS' CONTRIBUTIONS

ACV: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft. **PAL**: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **ISS**: Data curation, Methodology, Validation, Writing – review & editing. **IMB**: Data curation, Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. **MRNC**: Data curation, Investigation, Methodology.

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ORIGINAL ARTICLE

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Frequency of fibrosis in patients with incidentally detected hepatosteatosis

Muhammed Çiftçioğlu[1*], Bülent Kantarçeken[2], Murat Ispiroğlu[2], Kadir Gişi[2]

SUMMARY

OBJECTIVE: In this study, we aimed to elucidate fibrosis in patients who visited our outpatient clinic with complaints such as abdominal pain and dyspepsia and who had fatty liver by ultrasound imaging.

METHODS: A total of 119 patients who were admitted to the gastroenterology outpatient clinic of our institution with incidentally detected hepatosteatosis on ultrasound imaging were included in the study. Patients with hepatosteatosis were examined for fibrosis with the FibroScan-502-touch (Echosens, Paris, France) elastic tissue ultrasonography device. The effects of these parameters on hepatosteatosis and possible fibrosis degree were investigated.

RESULTS: No fibrosis was detected in 75 (63.02%) patients with hepatosteatosis on ultrasound imaging, 20 (10.05%) F1, 22 (18.48%) F2, 1 (0.8%) F3, and 0.1 (0.8%) F4. Accordingly, as the degree of steatosis increases in patients with incidentally detected hepatosteatosis, the degree and frequency of fibrosis increase with statistical significance ($p<0.05$). A statistically significant difference was found between the alanine transaminase increase and the hepatosteatosis degree ($p=0.028$). The median value of gamma-glutamyltransferase was 15 U/L in S0, 18.5 U/L in S1, 22 U/L in S2, and 26 U/L in S3 ($p<0.047$).

CONCLUSION: To date, no research exists on fibrosis in patients with incidental hepatosteatosis. The outcomes of this study elaborated that patients with hepatosteatosis in the community could be detected at least at an early stage by following up and diagnosing them with serum markers before they progress to end-stage fibrosis.

KEYWORDS: Non-alcoholic fatty liver disease. Liver cirrhosis. Fibrosis.

INTRODUCTION

Hepatosteatosis means that the liver has more than 5% of its weight as fat. Such patients are clinically observed as alcohol-related and non-alcoholic fatty liver disease (NAFLD). NAFLD is seen at an average rate of 25% across the world[1]. Most cases of NAFLD are asymptomatic. It is usually discovered incidentally with mild ALT elevation in routine blood tests or abdominal USG. NAFLD may progress to non-alcoholic steatohepatitis (NASH) associated with lobular inflammation and apoptosis, which may lead to hepatic steatosis, fibrosis, and cirrhosis[2].

Although liver biopsy is the gold standard diagnostic method for NASH patients, its use today could be more practical due to certain limitations. Therefore, less invasive, easily reproducible methods were considered, and a transient elastography device was developed with the trade name Fibroscan. This device is used to perform elastic tissue ultrasonography, which is a method that allows us to measure the elasticity of soft tissues with numerical data. This method simultaneously gives the patient's degree of fatty liver and the degree of possible fibrosis in the liver in light of numerical data[3].

Within the scope of this research, we aimed to elucidate fibrosis, which may have developed in patients who visited our outpatient clinic with complaints such as abdominal pain and dyspepsia and who had fatty liver by USG. In addition, we compared the hepatosteatosis data of both methods and devices by considering those who underwent Fibroscan for fibrosis or any other reason.

METHODS

A total of 119 patients who were admitted to the gastroenterology outpatient clinic of our institution with incidentally

[1]Kahramanmaraş Sütçü İmam University, Medical Faculty, Department of İnternal Medicine – Kahramanmaraş, Turkey.
[2]Kahramanmaraş Sütçü İmam University, Medical Faculty, Department of Gastroenterology – Kahramanmaraş, Turkey.
*Corresponding author: drmuhammedciftcioglu@gmail.com
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detected hepatosteatosis on USG were included in the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval was obtained from our institution, and informed consent was obtained from all participants.

Patients with hepatosteatosis were examined for fibrosis with the FibroScan-502-touch (Echosens, Paris, France) elastic tissue ultrasonography device. The routine biochemical values of the patients such as fasting blood glucose, fasting insulin, AST, ALT, GGT, platelet count, triglyceride, TSH, HDL, and LDL were processed. The body mass index (BMI) and body fat percentage were examined via the TANITA device. The effects of these parameters on hepatosteatosis and possible fibrosis degree were investigated.

Individuals under the age of 18 and above 65 years, pregnant women, patients with known liver diseases such as liver cirrhosis, drug use that may cause hepatosteatosis, diabetic patients, those with a BMI>40 kg/m^2, those with narrow intercostal range, those with active malignancy and congestive heart failure, and those with acid fluid in the abdomen were not included in the study.

Statistical analysis

Patient data collected within the scope of the study were analyzed with the IBM Statistical Package for the Social Sciences (SPSS) for Windows 23.0 (IBM Corp., Armonk, NY) package program. Frequency and percentage for categorical data and mean and standard deviation for continuous data were given as descriptive values. The "independent sample T-test" was used to compare two groups, and the "Pearson chi-square test" was used to compare categorical variables. The results were considered statistically significant when the p-value was less than 0.05.

RESULTS

A total of 119 patients were enrolled, of whom 52 (43.7%) were males and 67 (56.3%) were females. The average age of the patients is 39.15 years, and their average BMI is 29 kg/m^2 (Table 1).

According to the data obtained from the transient elastography examinations performed on the patients with hepatosteatosis, S0 indicates no fatty liver, S1 indicates first-degree fatty liver, S2 indicates second-degree fatty liver, and S3 indicates third-degree fatty liver. The population had a statistically significant difference between hepatosteatosis and advanced age, weight gain, increased BMI, and increased body fat ratio (p<0.001) (Table 2).

Table 1. Comparison of fibroscan and hepatosteatosis measurement with demographic characteristics.

		Fibroscan hepatosteatosis measurements (E-CAP)												p-value
		S0			S1			S2			S3			
		med	min	max	med	min	max	med	min	max	med	min	max	
Age (years)		30	20	49	29	19	65	40	24	62	45	19	80	<0.001
Height (cm)		159	151	177	171	154	185	166	151	188	161	139	183	<0.037
Weight (kg)		68.6	44.9	95.4	79.3	48.8	119.2	80.6	51.6	106.6	83.1	43.6	114.2	<0.001
BMI (kg/m^2)		26.3	16.4	37.7	27.0	20.1	36.0	29.4	20.9	38.7	31.4	17.7	42.2	<0.001
Fat (%)		26.9	4.1	53.5	24.1	13.3	39.4	34.6	14.9	47.6	35.6	12.1	49.4	<0.001
		n			n			n			n			
Gender	M	9.00			9.00			10.00			24.00			
	F	18.00			7.00			17.00			25.00			

Table 2. Comparison of ultrasound imaging and fibroscan hepatosteatosis measurements.

		Fibroscan hepatosteatosis measurements (E-CAP)									
		S0		S1		S2		S3		X^2	Kappa
		n	%	n	%	n	%	n	%	p-value	p-value
USG hepatosteatosis measurements	Grade 1	20	74.1	9	56.3	12	44.4	12	24.5	0.001	0.046
	Grade 2	7	25.9	7	43.8	13	48.1	28	57.1		
	Grade 3	0	0.0	0	0.0	2	7.4	9	18.4		

During the evaluation of hepatosteatosis by transient elastography of patients with hepatosteatosis detected on USG, 20 of 53 patients with grade 1 hepatosteatosis detected by USG did not detect steatosis by transient elastography. Accordingly, when the hepatosteatosis detection rates of USG and transient elastography are compared, although both devices do not agree in detecting low-grade hepatosteatosis, they seem more compatible in detecting advanced hepatosteatosis. This difference in compatibility may be due to the relative operator-dependent results of USG and the choice of cutoff values of the transient elastography device.

No fibrosis was detected in 75 (63.02%) patients with hepatosteatosis on USG, 20 (10.05%) F1, 22 (18.48%) F2, 1 (0.8%) F3, and1 (0.8%) F4. Accordingly, as the degree of steatosis increases in patients with incidentally detected hepatosteatosis, the degree and frequency of fibrosis increase with statistical significance ($p<0.05$) (Table 3).

The average fasting insulin in the group called S0 by Fibroscan was 7.9 mU/L, the average fasting insulin was 9.65 mU/L in S1 patients, the average fasting insulin was 12.7 mU/L in S2 patients, and the average fasting insulin in S3 patients was 16 mU/L. A statistically significant increase was observed between increased fasting insulin and hepatosteatosis ($p<0.001$).

The ALT average of the patients with S0 was 20 U/L, the average of those with S1 was 25 U/L, the average of those with S2 was 26 U/L, and the average of those with S3 was 34 U/L.

A statistically significant difference was found between the ALT increase and the hepatosteatosis degree ($p=0.028$). The edian value of GGT was 15 U/L in S0, 18.5 U/L in S1, 22 U/L in S2, and 26 U/L in S3 ($p<0.047$).

DISCUSSION

In this study, the frequency of liver fibrosis of various degrees and the parameters affecting fibrosis were investigated and discovered incidentally in patients not expected to have hepatosteatosis. Considering that obesity is a worldwide pandemic, early detection of fatty liver disease is important in terms of public health in order to prevent it from developing into an advanced liver disease such as cirrhosis. The prevalence of hepatosteatosis can be obtained even from the demographic characteristics of patients who visit outpatient clinics for any reason. Hepatosteatosis is seen more frequently in patients with advanced age, increased BMI, and increased body fat percentage[4].

Patients with chronic diseases such as diabetes mellitus and other conditions that may cause hepatosteatosis were not included in this study. Data such as age, gender, BMI, total fat mass and body fat percentage, lean body mass, and total body water were compared in a study of 253 patients with similar inclusion and exclusion criteria suggested by Lédinghen et al., who reported that the use of body composition parameters in NAFLD disease has a diagnostic value, which is similar to our research[5].

When USG detected hepatosteatosis, it was re-evaluated by transient elastography. Thus, the hepatosteatosis seen in USG could not be detected with transient elastography. This situation may be attributed to the evaluation of the operator performing the USG and the fact that the values measured with the transient elastography device have different reference intervals for each degree of hepatosteatosis. However, as seen in our study, as the degree of hepatosteatosis increased, the relationship between the degree of hepatosteatosis detection of USG and transient elastography increased[6]. Xu et al., compared USG, Fibroscan, and hepatic adiposity index (HSI) in a biopsy-based study of patients with chronic hepatitis B. In this study, the accuracy of Fibroscan and HSI was higher than ultrasound in the evaluation of mild and moderate hepatosteatosis shown in biopsy[7]. Macabuag-Oliva et al., found that the Fibroscan device was more sensitive than USG in detecting hepatosteatosis in a study conducted by 102 diabetic and metabolic syndrome patients[8].

Various degrees of fibrosis were detected in 44 (36.9%) of 119 patients with hepatosteatosis who were included in the study and had no condition to cause fibrosis. A multicenter study indicated that liver fibrosis was evaluated correctly with Fibroscan in patients with chronic viral hepatitis[9]. These data elaborated that individuals unaware that they have hepatosteatosis

Table 3. Fibrosis frequency according to ultrasound imaging grades.

		Fibroscan hepatosteatosis measurements (E-median)										p-value
		F0		F1		F2		F3		F4		
		n	%	n	%	n	%	n	%	n	%	
USG hepatosteatosis measurements	Grade 1	35	66.0%	10	18.9%	8	15.1%	0	0.0%	0	0.0%	0.032
	Grade 2	36	65.5%	6	10.9%	12	21.8%	1	1.8%	0	0.0%	
	Grade 3	4	36.4%	4	36.4%	2	18.2%	0	0.0%	1	9.1%	

in society develop fibrosis at a substantial rate. Our study diagnosed F4 fibrosis, and liver cirrhosis in one patient.

Fasting insulin, which is one of the biochemical parameters, is a part of the metabolic syndrome, which also includes obesity. In this study, when we compared fasting insulin with hepatosteatosis, it was observed that the degree of hepatosteatosis increased in patients with higher fasting insulin levels. Mikolasevic et al., conducted a prospective study of 648 patients on the effects of metabolic syndrome on fatty liver disease, and fibrosis and hepatosteatosis values were measured with Fibroscan. When the patients' insulin resistance was calculated by HOMA-IR and evaluated according to the degree of hepatosteatosis, a statistically significant positive correlation was found between insulin resistance and hepatosteatosis[10].

When the patients' mean GGT and ALT values were compared, a statistically significant increase was observed between the increase in GGT and ALT levels and hepatosteatosis. Serum ALT values were statistically significantly higher in individuals with hepatosteatosis. At the same time, serum ALT values increased statistically significantly in correlation with the increase in the liver's adiposity severity. In the GGT arm of the same study, serum GGT values were statistically significantly higher in all the three groups with mild, moderate, and severe fatty infiltration in the liver[11]. This allows us to speculate about hepatosteatosis by analyzing the serum parameters of patients who visited the outpatient clinic for any reason other than imaging.

CONCLUSION

To date, no research exists on fibrosis in patients with incidental hepatosteatosis. The outcomes of this study elaborated that patients with hepatosteatosis in the community could be detected at least at an early stage by following up and diagnosing them with serum markers, Tanita measurements, and transient elastography before they progress to end-stage fibrosis.

INSTITUTIONAL REVIEW BOARD APPROVAL

Ethics committee approval was obtained from Kahramanmaraş Sütçü İmam University of Medical Faculty on 16 October 2019 with protocol number 488665165-302.14.01.

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ETHICAL DECLARATION

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval was obtained from our institution. As this was a retrospective research, no informed consent has been obtained from participants.

AUTHORS' CONTRIBUTIONS

MÇ: Software, Supervision, Validation, Visualization, Writing – review & editing. **BK:** Software, Supervision, Validation, Visualization, Writing – review & editing. **MI:** Project administration, Software, Supervision, Validation, Visualization. **KG:** Formal Analysis, Investigation, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Does dietary total antioxidant capacity relate to oxidative stress levels in water immersion during labor? A case-control study

Tuğba Küçükkasap Cömert[1*], Seval Yılmaz Ergani[2], Meltem Uğurlu[3], Funda Akpınar[2]

SUMMARY

OBJECTIVE: The aim of this study was to investigate the effect of water immersion during the first stage of labor on maternal and neonatal oxidative stress and the association between serum and dietary total antioxidant capacity.

METHODS: Women were divided into two groups: those immersed in water during the first stage of labor (n=30) and those who had conventional birth (n=33). Total oxidative stress and total antioxidant status levels were examined in antepartum and postpartum maternal serum and neonatal cord blood samples. Dietary total antioxidant capacity was determined by the food frequency questionnaire.

RESULTS: Vitamin C and dietary total antioxidant capacity consumption were found to be higher in the water immersion group (106.92 mg/day and 18.94 mmol/gün, respectively) than the conventional birth group (92.69 mg/day and 15.99 mmol/gün, respectively) ($p<0.05$). Women immersed in water during the first stage of labor had lower total oxidative stress levels in antepartum and postpartum maternal serum and neonatal cord blood samples than those who had conventional birth (5.43±2.42 mmol/L and 5.59±3.35 mmol/L vs. 8.58±5.53 mmol/L and 12.68±16.58 mmol/L; $p<0.05$). Dietary total antioxidant capacity was found to be negatively correlated with total oxidative stress levels in antepartum and postpartum maternal serum and neonatal cord blood samples ($p=0.012$, $p=0.047$, $p=0.035$, and $p<0.05$).

CONCLUSION: Women immersed in water during the first stage of labor had lower total oxidative stress levels in their postnatal maternal serum and neonatal cord blood samples and dietary total antioxidant capacity was also a factor associated with low total oxidative stress levels.

KEYWORDS: Labor. Antioxidant. Oxidative stress.

INTRODUCTION

Pregnancy is a physiological process that increases tissue oxygen and metabolic demands. Increased oxygen demand leads to increased production of free oxygen radicals, leading to increased oxidative stress and lipid peroxidation in pregnant women compared to nonpregnant women[1]. The balance between oxidative and antioxidant systems plays a role in maintaining normal metabolic processes and preventing negative outcomes[2].

Water immersion during labor (WIDL) is a nonpharmacological conjugate of epidural analgesia. It is becoming a popular choice in contemporary obstetrics because it moves the birthing women from a passive role compliant of authority to an active participant in the whole event, giving a sense of achievement and satisfaction. Both the Royal College of Obstetricians and Gynecologists and the American College of Nurse–Midwives support water immersion in a healthy term "uncomplicated pregnancies"[3]. The 2018 Cochrane review states moderate- to low-quality evidence concerning water immersion during the first stage of labor on the mode of birth (spontaneous, instrumental and cesarean section) and no evidence for adverse neonatal outcomes[4].

The literature has investigated immersion practices during labor in terms of their effects on labor pain, anxiety, duration of delivery, and the newborn[5-7]. Also, studies have investigated the level of oxidative stress associated with immersion during labor and has been shown to be associated with lower oxidative stress levels. However, they have examined only postpartum values and attributed any differences in oxidative stress between groups to immersion during labor[8,9].

The present study investigated antepartum, postpartum, and neonatal TOS and TAS and evaluated dietary TAC of maternal diet. We hypothesized that WIDL is associated with lower TOS levels than CB and dietary TAC is a contributing factor to this situation.

[1]University of Health Sciences, Gülhane Faculty of Health Sciences, Department of Nutrition and Dietetics – Ankara, Turkey.
[2]University of Health Sciences, Etlik Zübeyde Hanım Training and Research Hospital – Ankara, Turkey.
[3]University of Health Sciences, Gülhane Faculty of Health Sciences, Department of Midwifery – Ankara, Turkey.
*Corresponding author: tugbaccomert@gmail.com
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METHODS

Participants

The study included pregnant women who met the following inclusion criteria: 18–35 years of age; primigravid; term pregnancy (37–41 weeks); no abnormal laboratory findings; no comorbidities; nonsmokers; spontaneous onset of labor; and women with singleton pregnancies, with newborns in vertex presentations and an estimated fetal weight of 2500–4000 g. Pregnant women were excluded if they presented with a ruptured membrane, had a body mass index (BMI) of ≥30 kg/m^2, or needed labor augmentation.

Sample size was calculated using Student's t-test with 0.80 power at a significant level of 0.05 as described by Sert et al.[8]. The calculation showed that samples should comprise a minimum of 60 pregnant individuals (30 per group) and their newborns.

Pregnant women who met the inclusion criteria were randomly divided into two groups: those immersed in water during the first stage of labor (n=30) (WIDL) and those who were on land during all stages of labor (n=33) (CB).

Data collection

Maternal and neonatal demographic and obstetric data were extracted from medical records, and data related to mothers' frequency of food intake in the antenatal period were collected via face-to-face interviews.

Water immersion conditions

Mothers in the WIDL group had their labors monitored in bathtubs, as described by Ibanoglu et al[9].

Biochemical analyses

Total oxidative stress and TAS levels were analyzed using a commercial enzyme-linked immunosorbent assay kit (Relassay, Turkey)[10,11]. The oxidative stress index (OSI) was calculated as the ratio of the TOS level to the TAS level according to the following formula: TOS (μmol H_2O_2 equivalent/L)/TAS (μmol Trolox equivalent/L). The OSI is an objective indicator of the balance between TOS and TAS levels[12].

Dietary analyses

To determine the daily nutrient intake (energy, macronutrient, and micronutrient intake), a semiquantitative food frequency questionnaire (FFQ) was used and analyzed via the Bebis version 7.2 software (Ebispro, Stuttgart, Germany)[13]. The dietary TAC of the participants was calculated using the antioxidant food database created by Carlsen et al.[14] based on the ferric-reducing antioxidant power assay.

Ethical approval

Approval from the ethics committee to conduct this study was obtained from the Clinical Research Ethics Committee of the Etlik Zubeyde Hanim Women's Health Training and Research Hospital (no. 2020/129, dated 09/09/2020). The study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of the country of origin.

Statistical analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 22.0 for Mac (SPSS Inc., Chicago, IL), and the data distribution was evaluated using the Kolmogorov-Smirnov test and found to be nonnormally distributed. The Mann-Whitney U test and multiple linear regression analysis were used. The level of significance was set at $p<0.05$.

RESULTS

There was no difference between the groups in pregnant women's mean age; gestational week; body mass index (BMI); cervical dilatation at the time of admission; estimated fetal weight; duration of the first stage of labor; and newborns' Apgar scores, birth weights, and neonatal intensive care status. The groups had similar sociodemographic and obstetric characteristics.

Women in the WIDL group had a higher intake of vitamin C (106.92±15.88 mg/day) and dietary TAC than women in the CB group (93.69±16.53 mg/day) ($p<0.05$) (Table 1).

Women who had CB had higher postpartum maternal serum TOS levels (8.58±5.53 μmol/L) and neonatal cord blood TOS levels (12.68±16.58 μmol/L) than those who were immersed in water during the first stage of labor (5.43±2.42 μmol/L, 5.59±3.35 μmol/L, and 0.02±0.01, respectively) ($p<0.05$) (Table 2).

In the WIDL group, dietary TAC was negatively correlated with antepartum ($p=0.000$) and postpartum ($p=0.014$) maternal serum and neonatal cord blood ($p=0.001$) TOS levels; moreover, it was negatively correlated with antepartum ($p=0.000$) and postpartum ($p=0.030$) maternal serum OSI levels ($p<0.05$). Also, dietary TAC was positively correlated with antepartum ($p=0.015$) and neonatal cord blood ($p=0.029$) TAS levels (Table 3).

DISCUSSION

In the current study, we found that postpartum maternal serum and neonatal cord blood TOS levels were found to be lower in the WIDL group than the CB group, and dietary TAC was correlated with antepartum and postpartum maternal serum and neonatal cord blood TAS levels.

Few studies in the literature have investigated the association between immersion during labor and oxidative stress[8,9,15]. From these studies, Sert et al.[8] investigated the serum levels of disulfide, disulfide/total thiol ratio, native thiol, total thiol, and albumin and Ischemia-modified albumin levels in neonatal cord blood. Ibanoglu et al.[9] examined the association between WIDL and oxidative stress based on the myeloperoxidase levels in cord blood samples. Assessment of the level of oxidative stress, measuring different oxidant and antioxidant molecules separately, is not recommended because it may cause overlapping and imprecise values as well as high costs; instead, TOS and TAS are advised to be more reliable, sensitive, and stable measures[10,11]. The method of measuring serum TAS and TOS levels in this study has high linearity, and the results are highly reproducible[11]. Also, Uzunlar et al.[15] used TAS and TOS levels as measures of oxidative and antioxidative stress in relation to immersion and examined these values only in cord blood samples postbirth; they attributed the difference in oxidative stress between the groups to immersion during labor. Our study, unlike the three studies mentioned above, determined antepartum serum TOS and TAS levels and found no difference between the groups. Thus, the present study clearly demonstrated that immersion was the only factor that resulted in low postpartum TOS levels in pregnant women of the WIDL group.

Diet has been the most important contributing factor for the regulation of oxidative stress levels[16]. Maternal dietary TAC (17.32±4.71 mmol/day) of women included in this study was found to be higher than those reported in a study from Brazil that evaluated the dietary TAC of 733 pregnant women (4.3 mmol/day)[17] but were similar to those reported in another study from Spain (17 mmol/day)[18]. The similarity of the data in the present study to that in the study conducted in Spain can be explained by the Mediterranean diet in both countries. The Mediterranean diet, owing to its abundance of fruits, vegetables, and oilseeds, is high in antioxidants[19].

It has been reported that dietary antioxidants, such as vitamin C, vitamin E, beta-carotene, and flavonoids, might reduce oxidative stress[20]. In our study results, vitamin C consumption was determined to be higher in the water immersion group than the CB group. This contributes to higher dietary TAC and lower TOS levels determined in the water immersion group.

Instead of analyzing each nutrient with antioxidant properties separately, we preferred to determine the dietary TAC, an indicator of the cumulative ability of diet antioxidants[20], effect on serum TOS and TAS levels. In addition, our study results showed that there was a relationship between dietary TAC and serum TOS and TAS levels in the WIDL group. In the CB group, a relationship (between dietary TAC and TOS levels) was shown only in the antepartum period. It is thought that this may be related to higher dietary TAC in the water

Table 1. Daily energy and nutrient intake of the water immersion during labor and conventional birth groups.

Variable	WIDL (n=30)	CB (n=33)	p
Energy (kcal/day)	1976.81±306.78	1980.33±386.95	0.863
CHO (g/day)	189.95±54.32	182.57±56.98	0.995
Protein (g/day)	75.38±16.52	78.97±17.69	0.457
Fat (g/day)	98.71±20.04	98.78±22.58	0.929
Fiber (g/day)	16.49±5.14	15.51±5.85	0.401
Vitamin A (µg/day)	837.14±600.46	820.24±580.69	0.815
Vitamin E (mg/day)	8.97±3.72	9.25±4.08	0.945
Vitamin B6 (mg/day)	0.78±0.27	0.86±0.30	0.227
Vitamin B12 (µg/day)	3.10±1.93	3.29±2.03	0.751
Folate (µg/day)	230.92±82.26	224.84±80.98	0.809
Calcium (mg/day)	545.56±197.59	541.41±20.61	0.929
Iron (mg/day)	6.71±2.45	7.21±2.69	0.530
Zinc (mg/day)	7.51±2.43	7.68±2.56	0.793
Vitamin C (mg/day)	106.92±15.88	92.69±16.53	0.045*
Dietary TAC (mmol/day)	18.94±5.78	15.99±4.13	0.048*

Dietary TAC: dietary total antioxidant status. *p<0.05.

Table 2. Biochemical findings in antepartum and postpartum maternal serum samples as well as neonatal cord blood samples.

Variable	WIDL (n=30)	CB (n=33)	p-value
Antepartum maternal serum			
TOS (µmol/L)	7.82±8.51	6.19±3.98	0.577
TAS (mmol/L)	2.15±0.21	1.91±0.31	0.121
OSI	0.66±0.36	0.38±0.16	0.690
Postpartum maternal serum			
TOS (µmol/L)	5.43±2.42	8.58±5.53	**0.047***
TAS (mmol/L)	2.15±0.37	2.04±0.17	0.308
OSI	0.61±0.59	0.34±0.18	0.074
Neonatal cord blood			
TOS (µmol/L)	5.59±3.35	12.68±16.58	**0.033***
TAS (mmol/L)	2.36±0.41	2.25±0.34	0.121
OSI	0.61±0.59	0.42±0.33	0.074

TOS: total oxidant status; TAS: total antioxidant status; OSI: oxidative stress index. *p<0.05.

Table 3. Multiple linear regression analysis of the effect of dietary total antioxidant capacity on antepartum and postpartum maternal serum and neonatal cord blood samples of total oxidant status, total antioxidant status, and oxidative stress index levels based on birth status.

Variable	Birth status	Beta	t	p	95% confidence interval	
Antepartum maternal serum						
TOS (μmol/L)	WIDL	-0.493	-4.452	0.000*	-0.490	-0.180
	CB	-0.430	-2.743	0.010*	-0.778	-0.113
TAS (μmol/L)	WIDL	0.470	2.604	0.015*	2.614	22.199
	CB	0.444	2.579	0.115	1.213	10.510
OSI	WIDL	-0.561	-4.092	0.000*	-10.552	-31.855
	CB	-0.512	-3.382	0.002*	-5.170	-20.992
Postpartum maternal serum						
TOS (μmol/L)	WIDL	-0.333	-2.626	0.014*	-1.418	-0.173
	CB	-0.091	-0.375	0.711	-0.439	0.303
TAS (μmol/L)	WIDL	0.159	1.138	0.265	14.311	4.111
	CB	0.264	1.179	0.248	8.019	2.156
OSI	WIDL	-0.351	-2.317	0.030*	-2.994	-0.170
	CB	-0.208	-1.437	0.163	-1.495	8.443
Neonatal cord blood						
TOS (μmol/L)	WIDL	-0.456	-3.589	0.001*	-1.236	-0.336
	CB	-0.277	-1.140	0.263	-0.193	0.055
TAS (μmol/L)	WIDL	0.397	2.315	0.029*	0.883	14.851
	CB	0.013	0.058	0.954	4.286	4.534
OSI	WIDL	-0.198	-1.309	0.202	-1.092	-4.921
	CB	-0.362	-0.2173	0.038*	-0.262	-8.635

TOS: total oxidant status, TAS: total antioxidant status, OSI: oxidative stress index. *p<0.05.

immersion group. Consumption of the recommended amount for dietary TAC has not been reported, and our findings suggest that there may be a threshold for interaction with serum TAS and TOS levels.

In conclusion, considering the relationship between dietary TAC and serum TOS levels, an increase in dietary antioxidant intake in the maternal diet can reduce both postpartum maternal serum and cord blood TOS levels.

CONCLUSION

This is the first study in which serum oxidative stress levels were determined in prenatal, postnatal, and neonatal cord blood, and their relationship with serum TAS and dietary TAC was evaluated in WIDL.

In this study and other studies, it has been shown that WIDL results in low serum TOS levels. However, in previous studies, dietary TAC, which highly affects serum TOS levels, has not been evaluated. According to our results, dietary TAC also contributes significantly to the provision of low TOS levels.

AUTHORS' CONTRIBUTIONS

TKC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **SYE**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **MU**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **NI**: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **FA**: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of oxytocin induction on early postpartum hemorrhage, perineal integrity, and breastfeeding: a case–control study

Yasemin Aydın Kartal[1], Leyla Kaya[2*], Saadet Yazıcı[3]

SUMMARY

OBJECTIVE: The aim of this study was to evaluate the postpartum hemorrhage, perineal integrity, and breastfeeding results of mothers who underwent oxytocin induction in the first stage of labor in the early postpartum period.

METHODS: This single-center observational case–control study was conducted in the obstetric unit of a public hospital in Istanbul. The study sampling included 44 pregnant women who received oxytocin induction (case group) and 44 pregnant women who did not receive oxytocin (control group). The Personal Information Form, LATCH Breastfeeding Assessment Tool, Breastfeeding Self-Efficacy Scale, Redness, Edema, Ecchymosis, Discharge, and Approximation Scale, and Postpartum Hemorrhage Collection Bag were used in data collection, and pad follow-up was carried out.

RESULTS: The amount of hemorrhage in the first 24 h of the postpartum period and the mean Redness, Edema, Ecchymosis, Discharge, and Approximation Scale score were significantly higher in the case group. While 47.7% of the oxytocin-induced women had 1st or 2nd, and 11.4% had 3rd or 4th degrees of lacerations, 20.5% of the control group had 1st or 2nd, and 2.3% had 3rd or 4th degrees of lacerations. There was no significant difference between the mean scores of the Breastfeeding Self-Efficacy Scale and LATCH Breastfeeding Assessment Tool in both groups.

CONCLUSION: According to the study findings, it was determined that oxytocin induction administered in the first stage of labor increased hemorrhage and perineal trauma in the early postpartum period but did not affect the results of breastfeeding.

Clinical Trial Registration Number: NCT04441125.

KEYWORDS: Breastfeeding. Oxytocin. Lacerations. Postpartum hemorrhage.

INTRODUCTION

Synthetic oxytocin (SynOT) is an essential component of the active management of labor. However, universal evidence-based standards regarding oxytocin dose and patient response are insufficient, and the decision to administer SynOT is mainly subjective[1]. Routine infusion of administered SynOT without evidence is of particular concern in terms of the risk of postpartum hemorrhage (PPH)[2]. Clinical studies of the effect of oxytocin administration during labor on the risk of PPH have reported conflicting results[3]. Khireddine et al. reported that labor oxytocin induction in low-risk women was associated with a higher risk of PPH than spontaneous vaginal delivery[4].

It has been reported that breastfeeding difficulties may be experienced in neonates due to exposure of the developing fetal brain, the protective blood–brain barrier of which is not yet mature, to high amounts of oxytocin[5]. Gomes et al. reported in their study that there is an association between intrapartum oxytocin dose and an increased risk of early cessation of breastfeeding among infants[6].

Perineal trauma is an essential complication in women after childbirth. In the literature, different study results show the effect of oxytocin administration on the perineum[7-9]. Similarly, in a study by Nakai et al. among Japanese women, severe perineal damage was found in 1.7% of 7,946 deliveries, and oxytocin induction was determined to be an essential risk factor, among other risk factors[10].

The almost routine use of oxytocin induction during labor in primigravid women in our country increases the importance of this issue. Determining how breastfeeding, which has an essential role in protecting and improving public health, PPH, the primary cause of maternal mortality, and perineal integrity are affected by oxytocin induction will provide essential data for health professionals and give an idea about the gaps in practice and research.

[1]University of Health Sciences, Faculty of Health Sciences, Department of Midwifery – İstanbul, Turkey.
[2]Zeynep Kamil Women and Children's Diseases Training and Research Hospital, Department of Obstetrics and Gynaecology – İstanbul, Turkey.
[3]Istanbul Health and Technology University, Faculty of Health Sciences, Department of Nursing – İstanbul, Turkey.
*Corresponding author: leylakaya02@hotmail.com

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The research conforms to the provisions of the Declaration of Helsinki (as revised in Brazil in 2013). All participants gave informed consent for the research, and their anonymity was preserved.

METHODS

This observational case–control study was conducted in the obstetric unit of a public hospital between September 2019 and December 2020.

The sample size was calculated using the volume of postpartum blood loss reported in the study by Mansy[5]. The minimum sample size was calculated at 80% power, a 5% significance level, and an expected effect size of 0.53 (Cohen's d), and it was found to be 44 in each group and 88 in total. The sample size was determined to be 44 patients for comparison of means between the two groups.

The inclusion criteria for pregnant women included primigravid pregnant women between the ages of 18 and 35 years, literate, with a hemoglobin of 10 g/dL and above, without mental or psychological problems or chronic diseases, and between 38 and 42 weeks of gestation. For newborns, those who had an Apgar score ≥7 at the first and fifth minutes, had no contraindications for breastfeeding, had no congenital anomalies, and had birth weight between 2500 g and 4000 g. Multiple pregnancies, a history of perineal surgery, and cases with breastfeeding problems related to the mother or the infant were excluded from the study.

Depending on the clinical decision to stimulate or increase the labor process, those who received an oxytocin infusion were defined as the case group. A total of 44 cases were randomly selected using computer-assisted randomization among the patients whose induction decision was made by the obstetrician working in the clinic (https://www.randomizer.org/#randomize). Pregnant women who did not receive an oxytocin infusion and were followed spontaneously formed the control group, and 44 pregnant women were randomly selected from this group using computer-assisted randomization.

Data collection tools

The Personal Information Form, the LATCH Breastfeeding Assessment Tool, Breastfeeding Self-Efficacy Scale (BSES), and PPH collection bag were used in data collection, and sanitary pad follow-up was carried out.

LATCH Breastfeeding Assessment Tool

The LATCH Breastfeeding Assessment Tool used to evaluate breastfeeding consists of five evaluation criteria. Each criterion is 10 points in total, to be evaluated in the range of 0–2 points. As a result of this measurement tool, the higher the score the mothers get, the higher their breastfeeding success. The Cronbach's alpha value for this study was determined to be 0.86.

Breastfeeding Self-Efficacy Scale

The scale, which has a 5-point Likert characteristic, consists of 14 items. A minimum of 14 points and a maximum of 70 points can be obtained, and higher scores indicate higher breastfeeding self-efficacy. The Cronbach's alpha value for this study was determined to be 0.82.

Redness, Edema, Ecchymosis, Discharge, and Approximation Scale

This scale covers five factors that indicate perineal wound healing: redness, edema, ecchymosis, discharge, and approximation. The sum of the scores obtained from the evaluation of the five categories creates the REEDA score. The lowest score is 0, while the highest score is 15. The highest score indicates severe perineal trauma.

Implementation of the research

Depending on the clinical decision to stimulate or increase the labor process, those who received an oxytocin infusion were defined as the case group. In contrast, spontaneously followed pregnant women who did not receive an oxytocin infusion formed the control group. The decision for oxytocin induction was made by the obstetrician in charge of the obstetric unit, independent of the study (there were three obstetricians in the clinic at the time of the study).

Oxytocin perfusion was created by diluting 5 IU of oxytocin into 500 mL of saline. Perfusion started with 4 mL/30 min of use. The oxytocin infusion was continued, doubling every 30 min until sufficient contractions were achieved (mean max: 76 mL/h). The fetal heartbeat monitoring was different in the oxytocin-administered and control groups—every 30 min in the former group, using electro-fetal monitoring (EFM), and every hour in the latter, using the EFM.

The amount of hemorrhage in the women was monitored during delivery with a PPH collection bag placed on the gynecological table. Women who gave birth generally in the institution where the study was conducted were discharged 24 h after delivery. The amount of bleeding until discharge was evaluated by weighing the pads and mattress protectors on a precision scale. The mattress protectors and pads used had standard sizes, and their dry weights were similar.

After birth, routine care was applied to the infants of the mothers in the oxytocin and control groups. As part of routine care, umbilical cords were clamped within 30–60 s as soon as the infants were born. Infants were placed under a radiant heater to receive standard care (vitamin K and hepatitis B vaccine application). Before discharge, mothers in both groups were given routine breastfeeding training by the clinic nurse.

The BSES and LATCH Breastfeeding Assessment Tool were applied to women in the case and control groups at the end of the postpartum 24th hour and 1st week. The 12th- and 24th-hour postpartum REEDA scale was applied.

The research data were analyzed in the SPSS 21.0 software. In the comparisons between the groups, when parametric assumptions were not provided for the quantitative variables, the Mann-Whitney U test was used in the independent sample constructs, and the Wilcoxon test was applied in the dependent constructs. Statistical analyses were performed using chi-square tests to compare women's characteristics between the two groups.

RESULTS

It was determined that the mean age of the women included in the study was 23.94±4.27 years, 47.1% of them were primary school graduates, by a majority, and the average gestational week was 39.10±0.98. There was no significant difference between the mean age, education level, gestational week, and prenatal hemogram (HGB) and hematocrit (HCT) counts of the pregnant women in the case and control groups (Table 1).

While it was determined that the first stage of labor was significantly shorter in the case group in which oxytocin

Table 1. Comparison of the characteristics of the groups.

	Case group (n=44)	Control group (n=44)	Tests/p
	Mean±SD	Mean±SD	
*First stage of labor total time (h)	3.24±2.97	8.63±4.16	[a]z=-5.246
			p=0.006
Second stage of labor total time (min)	33.88±13.94	20.44±8.93	[a]z=-4.210
			p=0.000
Third stage of labor total time (min)	9.80±4.93	6.85±2.99	[a]z=-2.775
			p=0.000
1-min Apgar scores	7.94±0.23	8.02±0.38	[a]z=-1.117
			p=0.264
5-min Apgar scores	9.20±0.47	9.65±0.48	[a]z=-3.634
			p=0.132
Prenatal hemogram	11.57±0.99	12.04±1.13	[a]z=-1.547
			p=0.122
Postpartum hemogram	10.04±1.42	10.64±1.08	[a]z=-1.387
			p=0.165
	**z=-4.840	**z=-5.090	
	p=0.000	**p=0.000**	
Prenatal hematocrit	35.19±2.72	36.64±3.05	[a]z=-1.933
			p=0.053
Postpartum hematocrit	30.96±3.65	32.74±3.08	[a]z=-1.592
			p=0.111
	**z=-4.890	**z=-5.087	
	p=0.000	**p=0.000**	
Postpartum blood collection bag (mL)	422.14±265.32	270.00±156.33	[a]z=-2.833
			p=0.005
Pad bleeding amount (mL)	172.00±67.42	127.14±38.69	[a]z=-3.022
			p=0.003
Total postpartum blood loss	594.14±277.19	397.14±175.47	[a]z=-3.350
			p=0.001

[a]Mann-Whitney U test. *The duration of the first stage of labor was calculated from the active phase.**Wilcoxon signed-rank test. Significant p-value are indicated in bold.

induction was administered, it was determined that the second and third stages of labor lasted significantly longer than the control group.

There was no significant difference between the weights and 1st and 5th-minute Apgar scores of newborns in the case and control groups. It was determined that 42.9% of newborns were females and 57.1% were males.

It was determined that there was no significant difference between the first evaluation (LATCH 24th hour) and the last evaluation (LATCH 1st week) in the case and control groups (Table 2).

When the mean BSES scores of the groups were examined, it was determined that there was no significant difference between the mean scores of the 24th-hour BSES and the first week in the case and control groups, while there was a significant difference between the first evaluation (24th-hour BSES) and the last evaluation (first-week BSES) in the case and control groups (Table 2).

When the perineal integrity of the groups was evaluated, it was determined that 47.7% of the women who underwent oxytocin induction had 1st or 2nd degree lacerations and 11.4% had 3rd or 4th degree lacerations. In the control group, 20.5% of the women had 1st or 2nd degree lacerations and 2.3% had 3rd or 4th degree lacerations.

While episiotomy was applied to 79.5% of the oxytocin group, it was determined that 75% of the control group

Table 2. Comparison of the groups' LATCH Breastfeeding Assessment Tool and Breastfeeding Self-Efficacy Scale score averages.

	Case group (n=44)	Control group (n=44)	Tests/p
	Mean±SD	Mean±SD	
Frequency of breastfeeding in the first 24 h	8.42±1.04	9.01±1.23	[a]p=0.623
LATCH Breastfeeding Assessment Tool 24th hour	8.91±1.63	9.14±1.84	[a]z=-1.047
			p=0.295
LATCH Breastfeeding Diagnostic Scale 1st week	9.26±1.04	9.28±1.63	[a]z=-0.922
			p=0.357
	[b]z=-1.615	[b]z=-0.607	
	p=0.106	p=0.544	
BSES 24th hour	60.50±8.21	61.45±6.77	[a]z=-0.268
			p=0.789
BSES 1st week	68.45±2.51	68.81±2.35	[a]z=-1.479
			p=0.139
	[b]z=-5.781	[b]z=-5.786	
	p=0.000	**p=0.000**	
REEDA 12th hour	2.77±1.73	0.54±1.12	[a]z=-5.323
			p=0.000
REEDA 24th hour	2.60±1.61	1.01±0.17	[a]z=-5.397
			p=0.000
	[b]z=-0.900	[b]z=-1.414	
	p=0.368	p=0.157	
	N	**n**	**Tests**
1st and 2nd degree lacerations	21	9	[c]X^2=13.585
			p=0.000
3rd and 4th degree lacerations	5	1	[d]X^2=0.055
			p=0.028
Episiotomy	35	33	[e]X^2=0.259
			p=0.611

[a]Mann-Whitney U test. [b]Wilcoxon signed-rank test. [c]Pearson's chi-square test. [d]Continuity (Yates) correction. [e]Fisher's exact test. BSES: Breastfeeding Self-Efficacy Scale; LATCH: LATCH Breastfeeding Assessment Tool. Significant p-values are indicated in bold.

underwent episiotomy, and no significant difference was found between the groups.

DISCUSSION

Routine administration of an oxytocin infusion without a valid indication based on evidence is of particular concern for the risk of PPH[2]. In the Grotegut et al.'s[4] study, oxytocin exposure was investigated in cases with blood transfusion, severe PPH, and PPH secondary to uterine atony. Women with severe PPH secondary to uterine atony have been reported to be exposed to significantly more oxytocin compared to the control group. In a population-based case (n=1483)–control (n=1785) study, the administration of SynOT during labor was reported to be an independent risk factor for severe PPH[11]. The research results support the literature, and it was determined that the amount of hemorrhage was significantly higher in the oxytocin induction group. The fact that the second stage of labor lasted longer in the case group compared to the control group explains the high amount of hemorrhage[12].

In the literature, there are different results of studies on the effects of oxytocin induction on the perineum[7,10,13]. Santos et al.[9] in their study with nulliparous women, determined that there was a significant relationship between the use of SynOT induction and perineal trauma. Similarly, Klokk et al.[13] reported that augmentation with oxytocin is an independent risk factor for obstetric anal sphincter injury in primiparous and multiparous women. Despite these findings, Oliveira et al.[7] in a retrospective study conducted in a public hospital, reported that no significant relationship exists between SynOT and spontaneous perineal lacerations. It has also been reported that this result may be related to other interventions, such as the oxytocin protocol, labor analgesia, and episiotomy. In this study, when the perineum was evaluated according to the REEDA scale and lacerations were considered, it was determined that perineal damage was significantly higher in the SynOT induction group. This study found that the implementation of episiotomy could not be evaluated objectively because in the hospital where the research was conducted, episiotomy is mainly preferred in primiparous mothers.

The literature states that SynOT induction administered before the onset of labor may affect breastfeeding[14]. Bai et al.[14] reported in their study on women living in Hong Kong that the duration of exclusive breastfeeding for the infants of mothers who received oxytocin induction was significantly lower than that of those who did not receive any intervention, and that induced labor shortened the duration of breastfeeding. In this study, it was determined that the breastfeeding status, breastfeeding frequency in the first 24 h, and breastfeeding self-efficacy were similar according to the LATCH scale of the mothers who had and did not receive oxytocin induction. The existing studies cannot reveal the effects of SynOT induction on breastfeeding, mainly due to the differences in oxytocin administration protocols. For this reason, healthcare professionals should be careful about breastfeeding difficulties in women and newborns receiving oxytocin.

Limitations

There were some limitations to the study, such as the fact that it was conducted in a single center and that PPH was evaluated within the first 24 h after delivery. In addition, the management of labor was carried out by three different physicians working in the clinic. This situation may affect many findings, such as the SynOT induction decision, episiotomy decision and repair, and perineal damage.

CONCLUSION

According to the study findings, it was determined that oxytocin induction administered in the first stage of labor increased hemorrhage and perineal trauma in the early postpartum period but did not affect breastfeeding outcomes. These results may provide healthcare professionals with a better understanding of the effects of oxytocin administration when making clinical decisions.

ETHICAL ASPECT OF THE STUDY

Zeynep Kamil Women and Children's Diseases Training and Research Hospital Clinical Research Ethics Committee permission (approval number: 76/2019.24.07) and institutional permission were obtained from the institution where the study was conducted.

AUTHORS' CONTRIBUTIONS

YAK: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **LK**: Conceptualization, Investigation, Methodology, Writing – review & editing. **SY**: Conceptualization, Investigation, Writing – review & editing.

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ORIGINAL ARTICLE

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Healthy lifestyle as predictors of common mental disorder during coronavirus disease

Laianne Liliane Pereira Troncha de Castro[1], Henrique Porcatti Walsh[2*], Marilita Falangola Accioly[3], Lislei Jorge Patrizzi Martins[3], Ana Carolina Otoni Oliveira[1], Lívia Pires Marra Graffitti[3], Maycon Souza Pegorari[3], Isabel Aparecida Porcatti de Walsh[3]

SUMMARY

OBJECTIVE: The objective of the present study was to verify the indication of common mental disorder and changes in healthy lifestyle among individuals affected by coronavirus disease, as well as to evaluate if changes in healthy lifestyle are predictors of common mental disorder.

METHODS: This descriptive, cross-sectional study employed an exploratory approach and quantitative methodology, using the Self-Reporting Questionnaire to assess the indication of common mental disorder and questions regarding healthy lifestyle during the pandemic.

RESULTS: A total of 280 individuals affected by coronavirus disease, aged 18 years and above, participated in the study. The average indication for common mental disorder was 5.0±5.34. The average age was characterized by adults (41.24±14.03 years), with the majority being women (57.9%), White (51.4%), and those in stable relationships (55.7%). Worsening sleep quality (β==6.327; p<0.001) was the main predictor of common mental disorder, followed by female gender (β==2.814; p<0.001) and worsening dietary habits (β==2.227; p<0.012).

CONCLUSION: These factors should be considered in the assessment of individuals affected by coronavirus disease to provide comprehensive care.

KEYWORDS: COVID-19. Healthy lifestyle. Mental health.

INTRODUCTION

The scenario of isolation, uncertainties, negative news from the media, and fear of falling ill, along with the economic, social, and environmental impacts caused by the coronavirus, has led to mental disorders[1], with anxiety and depression being common reactions, especially among those who were hospitalized due to concerns about their own health or that of others, the need for physical isolation, the potential risk of death, concerns about infecting others, or leaving family members who require care alone[2]. In this sense, a meta-analysis identified mental health disorders in affected populations, with prevalences of depression (15.97%), anxiety (15.15%), insomnia (23.87%), psychological stress (13.29%), and post-traumatic stress disorder (21.94%)[3].

Common mental disorder (CMD) is defined as a set of somatic, anxious, and depressive manifestations, such as memory and concentration difficulties, irritability, insomnia, fatigue, and feelings of worthlessness, affecting cognitive, physical, emotional, and behavioral functions[1]. It is a major public health problem as it increases the demand for and costs of healthcare services, directly interfering with the quality of life of individuals and their families[4].

However, adopting a healthy lifestyle, such as regular physical activity, a balanced diet, good sleep quality, and smoking and alcohol control, as well as family support and a good assessment of quality of life are essential for maintaining good mental health. However, during the coronavirus disease 2019 (COVID-19) pandemic, there has been a worsening of lifestyle habits, with an increase in behaviors that pose a risk to health[5].

Considering the recommendation of the Pan American Health Organization (PAHO)[6] regarding the provision of basic psychosocial and mental health support to those affected by COVID-19, questioning their needs, concerns, and conducting immediate assessments of anxiety, depressive symptoms, along with psychosocial support strategies and management of sleep-related problems, as well as the importance

Work carried out at the Federal University of Triângulo Mineiro (UFTM) – Uberaba/MG, Brazil. Address: Professor Aluízio Rosa Prata Research Center Building - Rua Vigário Carlos, No. 100, Abadia District, Uberaba - MG. Zip Code: 38025-350.

[1]Universidade Federal do Triângulo Mineiro – Uberaba (MG), Brazil.

[2]Medicine, Padre Albino Foundation – Catanduva (SP), Brazil.

[3]Universidade Federal do Triângulo Mineiro, Postgraduate Program in Physiotherapy – Uberaba (MG), Brazil.

*Corresponding author: rikewalsh@gmail.com

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of promoting healthy lifestyle for good physical and mental health, identifying possible relationships between changes in healthy lifestyle and CMD in individuals affected by COVID-19 can provide support to the healthcare system in addressing these issues, creating health education programs and activities that enable the restoration of healthy habits, considering comprehensive care.

The objective of the present study was to verify the indication of CMD and changes in healthy lifestyle among individuals affected by COVID-19, as well as to evaluate if changes in healthy lifestyle are predictors of CMD.

METHODS

Study design and sample

This was a descriptive, cross-sectional study with an exploratory approach and quantitative methodology, approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro (UFTM) under number 4647292.

Men and women aged 18 years or older who were affected by COVID-19 in a city in the interior of Minas Gerais, Brazil, participated in the study. The municipal health department provided the researchers with a list of notified individuals from March 1, 2020, to July 27, 2021, including personal data such as name, date of birth, and telephone number. Out of a total of 31,123 individuals aged 18 years and above, the sample size was calculated using the formula for simple proportion and finite population, with a margin of error of 10% and a confidence level of 95%, indicating a sample size of 201 affected individuals. A random selection was then performed to choose the participants.

Contact was made by telephone, following a standardized script and terms to be used in the approach, with three attempts made to reach each selected individual. During the call, when the participant agreed to participate in the research, he or she was informed that the call would be recorded. In the second step, if the participant did not want to answer by phone, he or she was invited to respond through a link provided via WhatsApp or email.

The participants were made aware of the Free and Informed Consent Form (FICF), and if they agreed to participate, they verbally expressed their agreement during the phone call. If they chose to respond directly through the provided link via WhatsApp or email, they downloaded the FICF and marked their acceptance on the form to proceed with the questionnaire.

Finally, participants were provided with an explanatory and illustrated booklet via WhatsApp or email, containing information on the topic, following recommendations from the World Health Organization (WHO) and the Ministry of Health.

During the data collection period (August 2021 to January 2022), a total of 1,214 calls were made. Out of these, 902 individuals were excluded, 32 declined to participate in the research, and 280 agreed to participate by phone or via WhatsApp, as represented in the flowchart in Figure 1.

Inclusion criteria were individuals diagnosed with COVID-19, registered by the municipality's health department, aged 18 years or above, who agreed to participate in the research after completing the FICF. Exclusion criteria were incomplete evaluation instruments and hospitalized or institutionalized individuals.

Variables and measures

Regarding sociodemographic aspects, age, gender, race, and marital status were considered. To assess the suspicion (presence or absence) of CMD, the Self-Reporting Questionnaire (SRQ-20) was used. This instrument, developed by the WHO for detecting symptoms and suggesting the level of suspicion (presence or absence) of minor mental disorders such as depression, anxiety, and stress, was adapted for national studies by Santos et al.[7] and is considered an easily applicable instrument, with the obtained scores related to the probability of CMD ranging from 0 (no probability) to 20 (extreme probability).

Changes in healthy lifestyle were assessed based on responses to questions about improvements, no changes, or worsening of physical activity, diet, smoking, alcohol consumption, and sleep during the pandemic.

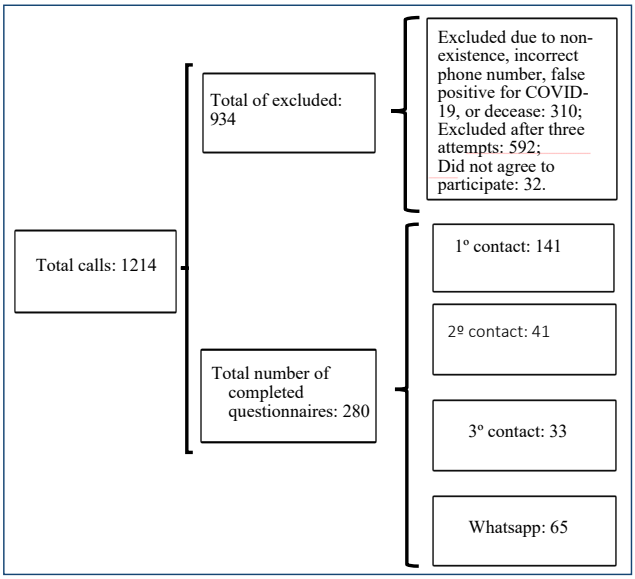

Figure 1. Flowchart of study participants. Source: Researchers' data, 2022. Minas Gerais, Brazil.

Data analysis

Descriptive statistical analysis was performed, including absolute and percentage frequencies, as well as means and standard deviations. The Mann-Whitney test was used to verify the associations between CMD and sociodemographic characteristics and changes in lifestyle habits, with a significance set at $p<0.05$. Linear regression analysis was conducted to evaluate the predictors of CMD using a 95% confidence interval (CI) and a significance level of 5%. The minimum prerequisites of normality, linearity, and homoscedasticity of residuals, as well as the absence of multicollinearity, were considered.

RESULTS

A total of 280 individuals affected by COVID-19, aged 18 years and above, participated in the study. The average indication for CMD was 5.0±5.34. The average age was characterized by adults (41.24±14.03 years), with the majority being women (57.9%), White (51.4%), and those in stable relationships (55.7%).

Being female and reporting worsening of physical activity, sleep quality, diet, and alcohol consumption were associated with higher average indications for the development of CMD ($p<0.05$), as presented in Table 1.

Multivariate analysis indicated that worsening sleep quality ($\beta==0.404$; $p<0.001$) was the main predictor of CMD, followed by female gender ($\beta=-0.311$; $p<0.001$), worsening diet ($\beta==0.172$; $p=0.002$), and worsening physical activity ($\beta==0.112$; $p=0.044$) (Table 2).

DISCUSSION

This study identified that worsening sleep quality was the main predictor of CMD, followed by female gender, worsening diet, and worsening physical activity.

During social isolation, there has been a significant impact on mental health, with poor habits, including an unbalanced diet, combined with the use of alcohol and tobacco, leading to sleep disorders and the onset of mental health problems[8]. Both the quantity and quality of sleep have been affected[9]. It is known that sleep is essential for human development and well-being. Thus, worsened sleep during the pandemic may also be associated with the development of other disorders.

Regarding diet, it should be considered that mental disorders are caused by failures in the communication of neurotransmitters with the nervous system, which affect psychomotor activities, appetite, sleep, and mood. Serotonin and dopamine are the main neurotransmitters associated with depression[10], and nutrition contributes to the production of these neurotransmitters, with nutrients serving as raw materials and regulating their quantities in the body. Vitamins, amino acids, and minerals are the most prominent nutrients in this process[11]. Additionally, stress triggers increased food consumption, particularly comfort foods, which can lead to significant changes in sleep and drive reward-seeking behaviors that increase the chances of uncontrolled eating[12].

As for female gender being a predictor of CMD, many studies have highlighted that women are more affected by the onset of mental disorders[13-16]. Being female has been associated with worsened mental health, especially in individuals affected by the virus when compared to those who were not infected[17]. In patients evaluated 3 months after the acute phase of the disease, it was noted that individuals with a psychiatric history and females may exhibit greater symptoms for the development of depression[18]. It is worth considering that Brazil already had one of the highest rates of mental health problems in the world even before the pandemic[19]. During the pandemic, women were found to have three times higher chances of developing mental disorders compared to men, as demonstrated by a study conducted in Rio Grande do Sul State[20]. Thus, appropriate attention should be given to this segment of the population.

One limitation of our study was its cross-sectional design, which limited our ability to establish a causal relationship between CMD and lifestyle habits. As a strength, we consider that it was conducted on a representative sample of the population affected by COVID-19 in the municipality.

Considering the need to assess the consequences of mental health in the care of clinical conditions, further studies should consider the importance of evaluating changes in healthy lifestyle in the population and their consequences for mental health. This would help establish public policies with measures focused on the mental health of those affected and those involved in primary healthcare and specialized outpatient care.

CONCLUSION

Worsened sleep quality, diet, physical activity, and being female were predictors for the development of CMD. These factors should be considered in the assessment of individuals affected by COVID-19 for comprehensive care.

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Table 1. Sample characteristics and distribution of means and proportions in groups according to the indication of developing a common mental disorder among individuals affected by coronavirus disease.

Variables	TMC code Mean±SD	p-value	Full sample n=280
Age (years)		0.715	
Sex			
Feminine	6.69±5.75	<0.001*	162 (57.9)
Masculine	2.69±3.65		118 (42.1)
Color			
White	4.79±5.31	0.396	144 (51.4)
Afrodescendant	5.23±5.38		136 (48.6)
Marital status			
Without union	5.23±5.18	0328	124 (44.3)
In union	4.83±5.48		156 (55.7)
Practice of physical activity			
Improved	4.05±4.82	<0.001*	43 (15.4)
Has not changed	3.45±4.24		129 (46.1)
Got worse	7.24±5.95		108 (38.6)
Food			
Improved	4.64±5.36	<0.001*	42 (15)
Has not changed	3.73±4.29		183 (65.4)
Got worse	9.53±5.08		55 (19.6)
Smoking			
Improved	6.83±5.42	0.056	6 (2.1)
Has not changed	4.78±5.19		263 (93.9)
Got worse	9.27±7.27		11 (3.9)
Alcoholism			
Improved	6.91±5.17	0.016*	22 (7.9)
Has not changed	4.60±5.06		243 (76.8)
Got worse	8.67±7.93		15 (5.4)
Sleep			
Improved	2.07±2.76	<0.001*	15 (5.4)
Has not changed	2.69±3.78		161 (57.5)
Got worse	9.01±9.32		104 (37.1)

CMD: common mental disorder; n: number of subjects; mean±standard deviation; *p<0.05, Mann-Whitney test. Source: Survey data. Uberaba-MG, 2022, n=280

Table 2. Common mental disorder and changes in lifestyle in people affected by coronavirus disease.

	TMC code					
	β	Standard error	T	p-value	Lower limits	Upper limits
Practice of physical activity	0.112	0.433	2.026	0.044*	0.024	1.731
Food	0.172	0.523	3.085	0.002*	0.584	2.647
Alcoholism	-0.033	0.772	-0.625	0.533	-2.004	1.039
Sleep	0.404	0.532	7.285	<0.001*	2.828	4.926
Sex	-0.311	0.574	-5.952	<0.001*	-4.544	-2.283

β: standardized coefficient; T: *t*-test; CI: confidence interval. *p<0.05. Source: Survey data. Uberaba-MG, 2022, n=280.

AUTHORS' CONTRIBUTIONS

LLPTC: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing review & editing. **HPW:** Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing review & editing. **MFA:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing review & editing. **LJPM:** Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing review & editing. **ACOO:** Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing review & editing. **LPMG:** Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing review & editing. **MSP:** Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing review & editing. **IAPW:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing review & editing.

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ORIGINAL ARTICLE

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Teaching of sexual medicine and gender issues in medical courses: students' perception

Alberto Trapani Junior[1], Manuela Silva Giordani[2*], Mariana Martins Notari[2]

SUMMARY

INTRODUCTION: In addition to reproductive purposes, human sexuality and sexual health are matters of great importance in the medical office. Despite this, there is still a deficiency in the training of Brazilian medical students regarding sexual medicine and gender issues.

OBJECTIVE: The objective of this study was to analyze the perception of fifth- and sixth-year students in relation to the teaching of sexual medicine and gender issues in medical courses.

METHODS: This is a descriptive and cross-sectional study with students from the last 2 years of medical schools in the State of Santa Catarina (internship classes), through the application of a self-administered, semi-structured online questionnaire.

RESULTS: A total of 164 students answered the questionnaire, with 83.5% (137/164) saying they had taken classes on sexual medicine and 47% (77/164) saying they had taken classes on gender issues. The participants judged the teaching inadequate in most of the topics addressed, and there was no significant difference between students from public and private schools. Notably, 79.9% (131/164) of the students considered the teaching of sexual medicine insufficient or inadequate, while 87.8% (144/164) considered the teaching of gender issues insufficient or inadequate.

CONCLUSION: The vast majority of students consider the teaching of sexual medicine and gender issues insufficient and inadequate.

KEYWORDS: Sexual health. Sexuality. Sexual and gender minorities. Students. Medical. Personal satisfaction.

INTRODUCTION

Sexual health was conceptualized in 1975 by the World Health Organization (WHO), which was included in the definition of reproductive health only 20 years later[1]. Beyond reproductive purposes and within the scope of human rights, the understanding of the dimension of human sexuality is fundamental[2]. The importance of this translates into the fact that most gender and sex minorities report of having experienced harassment and discrimination when seeking health services[3].

In a multi-country survey, it was shown that only 30% of medical schools include sexual health in the curriculum. When they do, the main focus is on reproductive biology and not on the diversity of behaviors and sexual expressions[4]. In Canada and the United States, only 22% of general practitioners regularly ask patients about their sexual health, making clear the gap in medical training[5].

In a Brazilian study, most students reported that the classes on sexual medicine were insufficient or nonexistent. Issues such as gender identity and expression, as well as sexual orientation are not addressed[6]. A survey with gynecologists, obstetricians, urologists, psychiatrists, and general practitioners showed that more than half of them do not regularly investigate the sexual health of patients, due to deficient knowledge in sexual health[7].

Countering the deficiency in medical education, there is an increase in complaints related to sexual dysfunctions among the population[8]. Among females, dysfunction of orgasm and sexual arousal are the most prevalent[9], and in males, erectile dysfunction is pointed out as predominant[10].

One strategy to improve sexual health care and care for sex and gender minority populations would be to improve academic training[11]. Assessing the level of satisfaction of medical students at the end of the course can be an instrument for a diagnosis of their training in this area.

This study aimed to analyze the perception of medical internship students regarding the teaching of sexual medicine and gender issues during the medical course.

METHODS

This is a descriptive, cross-sectional study. The study questionnaire was applied during the first semester of 2022 to students

[1]Universidade Federal de Santa Catarina, Universidade do Sul de Santa Catarina, Brazilian Federation of Gynecology and Obstetrics Associations, São José Regional Hospital Dr. Homero de Miranda Gomes, Palhoça Medicine Course.
[2]Universidade do Sul de Santa Catarina – Palhoça (SC), Brazil.
*Corresponding author: manuksg@gmail.com
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in the last 2 years of medical schools in the State of Santa Catarina, Brazil (internship classes).

The final sample size calculation was done using values from the mirror article[11], which referred to the outcome: "My college's current curriculum adequately prepared me to comfortably and competently serve sex and gender minorities," which were applied to OpenEpi at the 95% confidence interval plus 20% to have a margin of safety. A total of 154 questionnaires were then needed in order to have a satisfactory sample of the population.

The project was submitted to the Research Ethics Committee, and the questionnaires were applied after the Consent Opinion was released and approved under number 54431521.4.0000.5369.

Participants were approached by the researchers via electronic means. A link was sent that directed the participant to complete the questionnaire online, through the Google Forms platform. The participants were assured of secrecy and confidentiality. This is a self-administered, semi-structured questionnaire with open and closed questions. The self-answer instrument was applied in a single moment, and only one answer per participant was accepted. The participant could stop filling it out at any time, without being identified or suffering any embarrassment.

The dependent variable of the study was the teaching of sexual medicine and gender issues in medical courses. The independent variables included the types of teaching institution (public or private); subjects that offered these topics; topics covered in the classes; students' comfort and safety when dealing with sexual medicine and gender issues; and their satisfaction with the teaching of sexual medicine and gender issues.

The data were tabulated using descriptive statistics to obtain frequency distributions, with the use of the Windows Excel software. Later, the data were exported to the SPSS 16.0 program (IBM Corp., Armonk, NY, USA). Qualitative variables were described using absolute and relative frequencies, while quantitative variables were described as means and standard deviations for descriptive analysis. The chi-square (^{2}) or Fisher's exact test was used to test the homogeneity of proportions. The t-test for independent samples and the Mann-Whitney U test were used to compare parametric and nonparametric variables. The established significance level was $p<0.05$.

The study is based on Resolutions 510/2016 and 466/12 of the National Health Council, which, from the perspective of the individual and collectivities, incorporate bioethical references, such as autonomy, non-maleficence, beneficence, justice, and equity.

Before answering the questionnaire, the participant had to read and agree with the informed consent form. The researchers declare no conflict of interest.

RESULTS

A total of 164 completed forms were obtained [33 (20.1%) students from public universities and 131 (79.9%) from private universities]. Of the total of 1680 internal students contacted, 9.8% responded to the questionnaire. There was a prevalence of female participants (68.3%).

When asked about the teaching of sexual medicine, 83.5% (137/164) stated that they had been taught about this subject. There was no difference between the types of school (PR 1.02; 95%CI 0.87–1.20; p=0.820). As for gender issues, 47% (77/164) stated that this subject was addressed during medical school, and there was no difference between types of school (PR 1.12; 95%CI 0.77–1.64; p=0.557).

Table 1 shows which course subjects addressed the themes of this study. Gynecology, urology, and psychiatry stood out, and bioethics was the discipline that least addressed the theme. Gender issues were less addressed in all disciplines.

Table 2 shows that, in most of the topics covered in the questionnaire, the participants judged the teaching to be inadequate, in both private and public schools, and there are no differences between the types of school and the various specific topics.

When asked about patient care, they answered to feel comfort and security to attend: sexual minorities in 100/164 (61%); gender minorities, 63/164 (38.4%); cases of sexual dysfunctions, 75/164 (45.7%); questions of sexual practices with patients of minorities of sex or gender, 34/164 (20.7%); and questions of gender identity, 34/164 (20.7%).

As for the opportunities to attend cases involving sexual and gender minorities, 63% (104/164) of the students stated that they were insufficient or non-existent during the course.

Table 1. Distribution of the teaching of sexual medicine and gender issues according to disciplines.

	Sexual medicine n (%)	Gender issues n (%)
Gynecology	127 (77.4)	54 (32.9)
Urology	84 (51.2)	3 (1.8)
Infectology	9 (5.5)	2 (1.2)
Internal medicine	13 (7.9)	5 (3)
Psychiatry	25 (15.2)	18 (11)
Geriatrics	6 (3.7)	1 (0.6)
Endocrinology	17 (10.4)	10 (6.1)
Medical psychology	7 (4.3)	6 (3.7)
Bioethics	2 (1.2)	3 (1.8)
Public health	12 (7.3)	8 (4.9)
Other	5 (3)	8 (4.9)
Not addressed	25 (15.2)	87 (53)

Table 2. Comparison between public (n=33) and private (n=131) medical schools regarding perceived adequate teaching of sexual medicine topics and gender issues (total n=164).

Topic	Full n (%)	Public n (%)	Private n (%)	RP	95%CI	p
Sexual response cycle	69 (42.1)	15 (45.5)	54 (41.2)	1.103	0.55–1.69	0.660
STI prevention	154 (93.9)	32 (97.0)	122 (93.9)	1.041	0.96–1.12	0.410
Sexuality in special situations	47 (28.7)	11 (33.3)	36 (27.5)	1.213	0.69–2.12	0.506
Abortion	99 (60.4)	21 (63.6)	78 (59.5)	1.069	0.80–1.43	0.667
Sexual violence	86 (52.4)	18 (54.5)	68 (51.4)	1.051	0.74–1.49	0.786
Sexual and reproductive rights	52 (31.7)	7 (21.2)	45 (34.4)	0.618	0.31–1.24	0.147
Gender incongruence	27 (16.5)	6 (18.2)	21 (16.0)	1.134	0.50–0.82	0.766
Sexual orientation	28 (17.1)	8 (24.2)	20 (15.3)	1.588	0.77–3.28	0.221
Sexual dysfunction	91 (55.5)	20 (60.6)	71 (54.6)	1.101	0.80–1.51	0.565
Intersexuality	12 (7.3)	3 (9.1)	9 (6.9)	1.323	0.38–4.62	0.662
Effect of medications on sexuality	42 (25.6)	7 (21.2)	35 (26.7)	0.794	0.39–1.32	0.517
Effect of diseases on sexual response	31 (18.9)	4 (12.1)	27 (20.6)	0.588	0.22–1.56	0.266

PR: prevalence ratio; CI: confidence interval; STI: sexually transmitted infections.

In general, 79.9% (131/164) of students considered the teaching of sexual medicine insufficient or inadequate in their schools, while 87.8% (144/164) evaluated the teaching of gender issues insufficient or inadequate.

DISCUSSION

In 2014, the National Curriculum Guidelines (DCN) instituted the inclusion of gender and sexuality themes in medical curricula, explaining the need and importance of the approach of both[12]. Despite this, 8 years after the implementation of these guidelines, our study showed that there is still a lack in the teaching of these themes in the medical courses of SC, both public and private. This finding comes to add to similar studies carried out in other regions of Brazil[6,7] and in Europe[11-14].

When asked about the teaching of sexual medicine, 83.5% (137/164) said they had taken classes on this subject, and similar numbers were found in the study of Rufino et al. (95.2%)[7]. However, when it comes to gender issues, only 47% of students reported having taken classes on the subject. This disparity between the themes is in line with what was observed in the study by Zelin et al., where 92.7% of the participants felt comfortable treating sex minority patients, but only 31.7% felt comfortable serving gender minorities[11].

Analyzing the medical curricula of Brazil, we noticed that it is more focused on sexual medicine, leaving gender in the background[7,15,16]. Sexuality is considered to have a greater relationship with the physical body and stigmas of diseases that can be acquired by expressing it[16], leading to a "selective visibility" for the health care of this population with a focus on the genital organs, reproduction, and sexually transmitted infections (STIs), disregarding the complexity and subjectivity of these individuals[16], and perpetuating the cisnormative and binary pattern of the Brazilian health system, where minorities of sex and gender still suffer from the lack of competence, negligence, and frequent denial of their health rights[17].

According to the interviewees, the subjects that most addressed the theme of sexual medicine were gynecology (77.4%), urology (51.2%), and psychiatry (15.2%), while gender issues were most addressed only in gynecology (32.9%). Similar results were found in other studies[6,7,14], reiterating a punctual approach to these themes, focusing on reproductive and pathological themes or on "risk behaviors" related to these groups, such as cancer, abortion, psychiatric diseases, and STIs[7,15].

Reinforcing the aforementioned findings, the themes evaluated as having the most appropriate education were the prevention of STIs, abortion, sexual dysfunctions, sexual violence, and sexual response cycle, whereas the most inadequate were intersexuality, effect of acute and chronic diseases on sexual response, and gender incongruity. There was no statistical significance ($p<0.05$) among students from public and private schools. Similar findings were found in the study by Rufino et al[7].

Due to this reality, it is common for people who fit into minorities of gender and sex and/or with sexual issues in their broadest significance, to stop seeking medical care for fear of being treated badly, suffering discrimination and homophobia[3]. This leads to a greater risk of developing diseases and a consequent delay in diagnoses and initiation of treatments[18].

This contrast between the low teaching and approach of medical schools with a high practical demand for such professional skills makes us critically rethink the medical curricula not only in SC and Brazil but also in most medical schools around the world.

It was observed that, throughout the course, several topics were addressed in a timely manner, usually focusing on biological and pathological aspects and avoiding themes such as intersexuality.

The results indicate that medical schools should reassess the format of the teaching of sexual medicine and gender issues.

CONCLUSION

The findings presented here reinforce the lack in the teaching of sexual medicine and gender issues in the medical courses of Santa Catarina. The vast majority of students consider the teaching of sexual medicine and gender issues insufficient and inadequate.

AUTHORS' CONTRIBUTIONS

ATJ: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **MSG**: Conceptualization, Data curation, Formal Analysis, Investigation, Writing – review & editing. **MMN**: Data curation, Formal Analysis, Investigation, Writing – review & editing.

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ORIGINAL ARTICLE

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Prevalence of eating disorders in patients with celiac disease: a comparative study with healthy individuals

Renato Nisihara[1,2*], Ana Clara Maier Techy[1], Carolina Staichok[1], Thais Carolini Roth[1], Grácia Furiatti de Biassio[1], Luani Risso Cardoso[1], Lorete Maria da Silva Kotze[2]

SUMMARY

INTRODUCTION: Celiac disease is a chronic immune-mediated disease, which is triggered and maintained by gluten in genetically susceptible individuals. Eating disorders are a persistent disturbance in eating-related behavior that results in altered food consumption or absorption and that significantly impairs physical health or psychosocial functioning.

OBJECTIVE: This study aimed at evaluating the prevalence of eating disorders in Brazilian celiac patients.

METHODS: This cross-sectional study was conducted as online survey including adult celiac patients who agreed to participate and a paired control health group. Questionnaires included questions about socioeconomic data and celiac disease diagnosis, and a validated questionnaire about eating disorders (Eating Attitudes Test-26.

RESULTS: In total, 741 responses were studied, with 484 from the celiac group and 257 from the control group. No significant difference was observed between the number of individuals at risk of developing eating disorder (p=0.39). Both groups showed a high risk of developing eating disorders (34.2% in the celiac group and 37.7% in the control group). Furthermore, among patients with celiac disease, we found higher scores on the Eating Attitudes Test-26 in those with depression (p=0.0013), those with living difficulty due to the disease (p<0.0001), and those dissatisfied with their weight (p<0.0001).

CONCLUSION: In the sample analyzed, no greater risk of eating disorders was identified in patients with celiac disease compared with the control group. However, in general, about one-third of the respondents in each group had scores associated with the risk of eating disorders. Among celiac patients, depression, difficulties living with celiac disease, and being unhappy with one's weight were associated with higher risk for eating disorder.

KEYWORDS: Celiac disease. Eating disorders. Anorexia. Bulimia. Binge eating disorder. Depression.

INTRODUCTION

Celiac disease (CD) is a chronic, systemic, and immune-mediated disease that happens from exposure to gluten present in food, mainly affecting the small intestine and generating gastrointestinal and non-gastrointestinal symptoms[1]. Due to the accessibility of the diagnosis, its incidence has increased globally[2,3]. The treatment consists of a gluten-free diet (GFD), which improves the symptoms, avoiding complications[4]. Both symptomatology and dietary restriction negatively interfere with the patients' quality of life. Some authors have reported that much longer the time since diagnosis and GFD is, the more adapted the patients are[3].

Eating disorders (EDs) are a persistent disturbance in eating or eating-related behavior that results in altered food consumption and that significantly impairs physical health or psychosocial functioning[5]. Among these disorders, anorexia nervosa (AN), bulimia nervosa (BN), and binge ED (BED) are classified as specific EDs. These diseases are characterized by continuous disturbances in eating, different weight control modalities, and exacerbated care with aesthetics/body weight[6]. According to the WHO, the estimated prevalence of ED is 4.7% in the general Brazilian population[7]. Is it possible that the need to restrict the diet of celiac people is capable of generating ED? Studies carried out in the United States, Sweden, and Poland showed a higher frequency of ED in celiac patients[8]. In the Spanish population, there is no significant difference in the prevalence of ED between celiac patients and people in general[9].

People with CD must strictly avoid gluten-containing foods to manage their condition effectively. This restrictive diet can lead to a heightened focus on food, eating habits, and body weight. In some cases, this can manifest as disordered eating patterns, such as binge eating, purging, or extreme dietary restrictions, which are the characteristics of various EDs[8,9]. There is a genetic association between the immunoregulatory

[1]Universidade Positivo, Department of Medicine – Curitiba (PR), Brazil.
[2]Universidade Federal do Paraná, Clinical Hospital – Curitiba (PR), Brazil.
*Corresponding author: renatonisihara@gmail.com, renatonisihara@up.edu.br
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mechanisms of CD and common metabolic pathways for diabetes and AN, favoring the coexistence of these chronic diseases[10]. Additionally, there was a similarity in the epidemiological profile of celiac patients with those with ED, the majority of whom were women, Caucasian, and young[11].

In Brazil, no studies were found on the frequency of ED among patients with CD. Thus, we aimed to investigate the frequency of ED in celiac patients, comparing it with the non-celiac population. Additionally, we aimed to assess whether socio-demographic or CD-related factors are associated with a greater possibility of ED.

METHODS

Ethical issues

This is an analytical cross-sectional study.

The study was approved by the Research Ethics Committee (CEP) under number 4770592. All volunteer research participants signed an informed consent form agreeing to participate.

Samples

The participants, self-declared as diagnosed with CD, were invited through support groups for celiac patients, whether belonging to celiac associations pages on Facebook, Instagram, and websites of associations that support celiac patients such as Acelpar (Associação de Celíacos do Paraná) and Fenacelbra (Federação Nacional das Associações de Celíacos do Brasil). Data were collected via online questionnaire from August 2021 to April 2022.

For the healthy control group, the questionnaire was sent by WhatsApp groups, e-mail, or Instagram. Participants in the control group declared that they did not have any chronic disease, especially DC. The two groups of participants were matched by sex and age.

For both groups, duplicate, inconsistent, and incomplete responses were excluded.

Data collection

An online questionnaire was used in Google Forms format, which was configured in three sections. Participants in the control group only answered questions in sections 1–3.

Section 1: Demographic data: sex, age, schooling or level of education, and ethnicity.

Section 2: Data on CD diagnosis, symptoms; which difficulties related to GFD; and whether the GFD had professional guidance. Family history of CD, questions about the patient's weight, and depressive symptoms.

Section 3: Validated questionnaire Eating Attitudes Test-26 (EAT-26) in Portuguese. The EAT-26 has been used widely to measure the cognitive and behavioral symptoms of disordered eating in clinical and general population comprising males and females[12], and it had 26 questions in the form of a Likert scale of points in which each answer has a value. The score is calculated from the sum of answers for each item, ranging from 0 to 78 points. Scores greater than 21 points are considered indicative of risky eating behavior, analyzing variables of bulimia, weight, body image, and psychological symptoms. EAT-26 is considered a reliable and valid instrument[12].

Statistical analysis

Statistical analyses were performed using the Graph Pad Prism 7.0 program. The Shapiro-Wilk test was applied to assess data normality. Continuous variables were expressed as mean and standard deviation or median and interquartile range (IQR) and compared using the non-parametric t-test or Mann-Whitney U test, as appropriate. Categorical variables were expressed as percentages and compared using Fisher's exact test or chi-square test, as appropriate. $p<0.05$ were considered statistically significant.

RESULTS

A total of 835 responses were collected, of which 94 were excluded. Thus, 741 participants were studied, with 484 from the celiac group and 257 from the control group.

Demographic data

The analysis of the sociodemographic data is available in Table 1. The average age of the studied patients was 38.1±3.8 years and that of the control group was 34.1±5.2 years.

It was observed that there was no difference in the number of women who answered the questionnaire between the groups, as well as in schooling/education and ethnicity. However, the number of male respondents in the control group was significantly higher ($p=0.002$).

Data about celiac disease

These questions were answered only by celiac patients and the data are available in Table 2. In the sample, 32.8% (159/484) had a family member with CD. Among them, 258 (53.3%) were satisfied with their weight. Regarding the diet, 86.1% (417/484) of the participants declared adherence to the GFD. Social networks were the most cited sources of information. Among those who complied with the GFD, 94% reported improvement in symptoms. Regarding feelings about having

Table 1. Demographic data of celiac patients and healthy controls.

Celiac patients (n=484)			Controls (n=257)	p
Age (years)	<18	0	0	0.31
	18–20	12 (2.4%)	17 (6.6%)	
	21–30	132 (27.2%)	114 (44.3%)	
	31–40	161 (33.2%)	41 (15.9%)	
	41–50	108 (22.35)	37 (14.3%)	
	51–60	51 (10.5%)	34 (13.2%)	
	>60	20 (4.1%)	14 (5.4%)	
Gender	Female	458 (94.6%)	213 (82.8%)	0.43
	Male	26 (5.4%)	44 (17.1%)	0.002
Schooling	Primary education	5 (1%)	2 (0.7%)	0.152
	High school	61 (12.7%)	41 (16.2%)	
	Incomplete high school	71 (14.6%)	100 (38.9%)	
	Graduation	134(27.6%)	61 (23.7%)	
	Postgraduation	217 (44.8%)	55 (21.4%)	
Ethnicity	Yellow	10 (2%)	13 (5%)	0.66
	White	408 (84.2%)	216 (84%)	
	Afrodescendants	66 (13.6%)	28 (10.9%)	

CD, 286 (59%) said they lived well with the disease, 155 (32%) declared difficulties, and 42 (8.6%) had many difficulties in living with CD.

Regarding the diagnosis of mental disorders, 120/484 (24.7%) had a diagnosis of depression, and 116 (23.9%) were treated for depression. Among participants with DC, 11 (2.2%) reported having anorexia, 12 (2.4%) bulimia, and 11 (2.2%) BED.

Among the 118 (24.3%) patients with CD who reported of having depression, the EAT score had an average of 20 points (IQR=13–26), and in the 362 (74.7%) who said they did not have depression, the average was 16 points (IQR=11–23; p=0.0013), as can be seen in Figure 1a. Among the 285/484 (58.8%) participants with CD who reported living well with the disease, the EAT scores had a median of 15 points (IQR=11–23). Among the 197 (38.6%) who reported not living well with DC, the median was 19 points (IQR=14–26), which was significantly higher (p<0.0001), as shown in Figure 1b. Of the participants with CD, 225 (46.4%) reported that they were not satisfied with their weight and had EAT scores with an average of 19 points (IQR=14–26). Among the 257 (53%) who reported being happy with their weight, an average of 15 points was observed (IQR=10–22; p<0.0001), as shown in Figure 1c.

Data about Eating Attitudes Test-26

The average EAT score in patients with CD was 17 (IQR=12–25), and that of the control group was 18 (IQR=11–26; p=0.32). According to the classification of the scores obtained in the EAT-26 questionnaire, among celiac patients, 166/484 (34.2%) were at risk for developing ED, of which 157/458 (34.3%) were women. In celiac men, 9/26 (34.5%) were at risk for developing ED (p=0.35). In the comparison group, 97/357 (37.7%) people were at risk, with 86/213 (40.3%) being women. Of the men's group, 11/44 (25%) were found to be at some risk for developing ED (p=0.22). Schooling and age did not significantly influence EAT scores in the study sample (p=0.43 and p=0.38, respectively).

Comparing the two groups, celiac and control, no significant difference was observed between the number of individuals at risk for developing ED (p=0.39) and number of those who were not. Additionally, there was no difference between celiac women and controls (p=0.14) and between men in the celiac and control groups (p=0.42).

Among 34 celiac patients who said they had a diagnosis of ED, 23 (67.8%) had scores above 21 points. These data were not evaluated in controls, as having a diagnosed ED was an exclusion criterion.

DISCUSSION

Although CD and ED are topics much discussed separately in the literature, there are few studies that associate these two diseases. Initially, the authors had the hypothesis that the dietary

restriction imposed on celiac patients could increase the risk for ED. There are no reports on this topic in the Brazilian population. The way the investigation was carried out (online) provided a large sample (484 celiac patients) and the results did not demonstrate a greater risk for the celiac group when compared with the control group. On the contrary, our data indicated, in both studied groups, that about one-third of the participants had a risk score for developing ED.

Table 2. Clinical data of celiac patients studied (n=484).

		n (%)
Has a family member with CD? (n=484)	Yes	159 (32.8)
	No	323 (66.7)
Are you happy with your weight?	Yes	258 (53.3)
	No	226 (46.6)
Consume food with gluten?	>1x/week	8 (16.5)
	1x/week	21 (4.2)
	1x/month	30 (6.1)
	Eat without restrictions	6 (1.2)
	Never	417 (86.1)
How to guide yourself on the diet?	Instagram	97 (20.2)
	Sites	121 (25.1)
	Books	23 (4.7)
	Other	243 (50.2)
Improved symptoms with diet?	Yes	455 (94.4)
	No	17 (5.6)
How does it feel to have CD?	Live well	287 (59.0)
	I have difficulties	155 (32.0)
	I have many difficulties	42 (8.6)
Has a diagnosis of depression?	Yes	120 (24.7)
	No	362 (74.7)
Get treatment for depression?	Yes	116 (23.9)
	No	366 (75.6)
Has a diagnosis of eating disorders?	Anorexia	11 (2.2)
	Bulimia	12 (2.4)
	Binge eating disorder	11 (2.2)
	No	443 (91.5)
	Did not answer	7 (1.4)

Most of the participants in this study were females and educated. In general, women have better adherence (>80%) to online questionnaires compared with men[13]. Additionally, DC affects a greater number of women[8]. There were no significant differences in the answers between genders in both groups in the topics studied.

Two articles that correlate both diseases were found in the European literature. The parameter used in their risk assessment method was the EAT-26 questionnaire, which has a sensitivity of 82% and is efficient for screening ED[14]. However, they showed different results. A study carried out in Italy included adult patients with untreated CD and a possible association of this group with behaviors suggestive of ED. The data presented indicated that the behavior of ED was higher in patients with CD and in women[12]. Our study obtained a different result, showing that there was no association between ED and CD. Another study in Spain obtained a similar result to our data, with no significant difference between controls and celiac patients[9].

It is suggested that patients with CD have a more restricted diet, leading them to have more guidance on nutrition and health care.

From the responses obtained on the questionnaire directed only at patients with CD, 94% of them improved their symptoms with the GFD. Probably, with the improvement of gastrointestinal symptoms and disease control, these patients had a slight weight gain. When asked if the celiac participant was satisfied with their weight, almost half of the sample was not happy with their body weight. In that same sample, more than 90% denied any type of ED. However, according to the EAT assessment, 34.2% are at risk for developing one of these disorders. It is suggested that probably these patients are underdiagnosed.

For participants with CD diagnosed with depression, difficulties in living with CD and being dissatisfied with their weight are

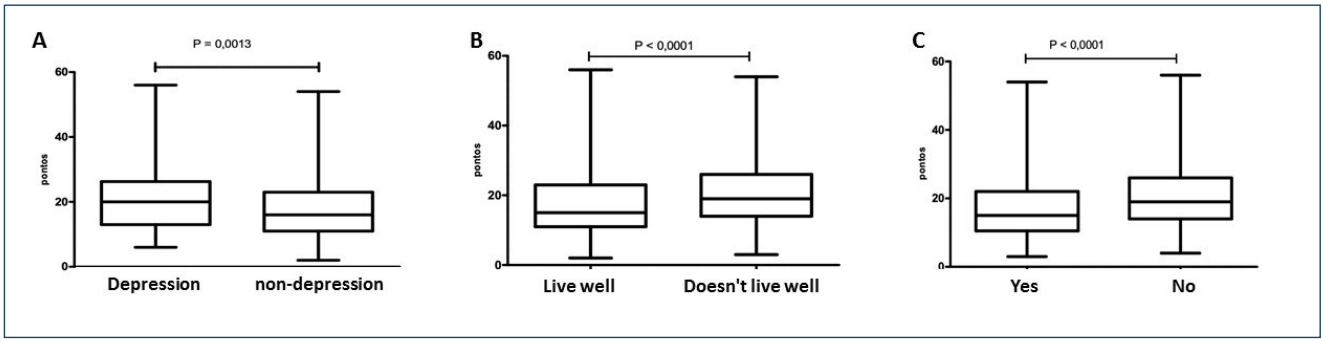

Figure 1. (A) Eating Attitudes Test-26 scores in celiac patients with and without depression. (B) Eating Attitudes Test-26 scores in patients who live well or not with celiac disease. (C) Eating Attitudes Test-26 scores in celiac patients who are or are not happy with their weight.

significantly associated with higher EAT scores. Therefore, they are at greater risk for developing ED. Other studies show that, although patients with depression face symptoms of ED, they do not meet the DSM criteria to be diagnosed as such. However, patients with BED are associated with a higher risk of suicide[15].

Furthermore, it can be observed that 67.8% of patients with CD who claimed to have a diagnosis of ED showed a high score on the EAT-26. It is possible that they are not being adequately treated for AT.

This study has some limitations related to its cross-sectional design and the data collection method used. The survey was conducted online, without access to medical records of patients with CD, which may lead to inaccuracies. However, we sought to disseminate the survey questionnaire also in groups of patients previously diagnosed with CD, in order to avoid the responses of people without the disease. The high-risk index observed in both groups may be associated with the fact that people who are already at risk for developing ED may be more interested in answering a questionnaire on the subject, creating a selection bias. In addition, disclosure was mainly via social networks and some patients did not have access to the form. On the contrary, the online survey allows for the participation of a greater number of people and does not cause embarrassment due to its anonymous character, making the answers more reliable.

In conclusion, there was no greater risk for celiac patients to develop ED compared with the control group. However, for both groups, attention was drawn to the high frequency of people at risk for having one of the types of ED, given that one-third of respondents are at risk in both groups. Depression, difficulties in living with CD, and being dissatisfied with one's own weight are significantly associated with higher EAT scores, suggesting a greater chance of developing ED.

ETHICS APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Committee of Ethics in Research from Positivo University under protocol number 4770592.

CONSENT TO PARTICIPATE

All participants signed an informed consent.

CONSENT FOR PUBLICATION

Yes.

TRANSPARENCY DECLARATION

The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

AUTHORS' CONTRIBUTIONS

ACMT: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **CS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **TCR:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **GFB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **LRC:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **RN:** Conceptualization, Formal Analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **LMSK:** Conceptualization, Formal Analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Vitamin D levels in patients attending a tertiary care hospital in Mogadishu, Somalia: a retrospective review of 28,125 cases

Mosab Ahmed Nor[1], Esra Keles[2*], Mohamed Abdulkadir Hassan-Kadle[3], Mohamed Abdulahi Hassan[4], Kursad Nuri Baydili[5], Hasan Huseyin Eker[6,7]

SUMMARY

OBJECTIVE: The objective of this study was to identify the prevalence and risk factors for vitamin D deficiency among patients attending a tertiary hospital in Mogadishu, Somalia.

METHODS: This retrospective study examined the results of serum 25-hydroxy-vitamin D tests of 28,125 patients admitted to Somalia Mogadishu-Turkey Training and Research Hospital between January 2017 and December 2021. Vitamin D insufficiency is defined as 20–30 ng/mL, deficiency as 10–19 ng/mL, and severe deficiency as <10 ng/mL.

RESULTS: A total of 28,125 patients with a mean age of 44.27±20.4 years were included in the study. The majority of patients were in the age group of 19–40 years. The mean serum level of 25-hydroxy-vitamin D was 28.42±15.34 ng/mL. Of the patients included in the study, 5.8% (1,618/28,125) had vitamin D sufficiency, 6.5% (1,826/28,125) had vitamin D insufficiency, 41.8% (11,761/28,125) had vitamin D deficiency, and 45.9% (12,920/28,125) had severe vitamin D deficiency. The mean serum 25-hydroxy-vitamin D levels were lower in females than in males (p<0.001).

CONCLUSION: The study indicated a high prevalence of vitamin deficiency among patients attending the largest tertiary care hospital, particularly female patients and older people. It is recommended to develop educational and awareness programs, and campaigns to reduce vitamin D deficiency in the population, especially those at high risk.

KEYWORDS: Vitamin D. Vitamin D deficiency. Somalia.

INTRODUCTION

Vitamin D deficiency is one of the major public health problems affecting over 1 billion people worldwide[1]. Vitamin D is essential for every individual to lead a healthy life. Vitamin D is a prohormone and fat-soluble vitamin. Even though a small amount of vitamin D is obtained from food, the major amount of vitamin D is obtained from exposure to the ultraviolet-B (UVB) component of sunlight[2].

There is an ongoing debate over the determination of serum 25-hydroxy-vitamin D (25(O.H.)D) concentration for defining the vitamin D status[1,3]. Additionally, the vitamin D range varies between populations and depends upon many factors. Therefore, the Scientific Advisory Committee on Nutrition (SACN) has recently contributed an excellent overview of the current vitamin D status as well as defined recommendations for adequate 25(O.H.) D concentrations in the general population[4].

Circulating 25(O.H.) D is a reliable indicator of vitamin D nutritional status. Recently, many studies have used 30 ng/mL as a cutoff value, and most experts now recommend ≥ff ng/mL as the normal level of 25(O.H.) D , 20–29 ng/mL as vitamin D insufficiency, and ecomng/mL as vitamin D deficiency[5].

Many studies have shown that inadequate levels of vitamin D can lead to a variety of negative health conditions, from rickets and osteoporosis to obesity, type 2 diabetes, hypertension, depression, fibromyalgia, and Parkinson's disease[6,7]. Vitamin D deficiency may even contribute to the development of cancers as well as cardiovascular disease, Alzheimer's disease, stroke, autoimmune diseases, pelvic floor diseases, and periodontal disease[7].

[1]University of Health Sciences Turkey, Mogadishu Somalia-Turkey Recep Tayyip Erdoğan Training and Research Hospital, Department of Internal Medicine – Mogadishu, Somalia.
[2]University of Health Sciences Turkey, Kartal Lütfi Kırdar City Hospital, Department of Gynecologic Oncology – İstanbul, Turkey.
[3]Abrar University, College of Medicine and Health Science, Center For Digestive and Liver Diseases, SomGastro Clinic – Mogadishu, Somalia.
[4]SIMAD University, Dr. Sumait Hospital, Faculty of Medicine and Health Sciences, Department of Internal Medicine – Mogadishu, Somalia.
[5]University of Health Sciences Turkey, Hamidiye Faculty of Medicine, Department of Biostatistics – İstanbul, Turkey.
[6]University of Health Sciences Turkey, Hamidiye Faculty of Medicine, Department of Public Health – İstanbul, Turkey.
[7]University of Health Sciences Turkey, Mogadishu Somalia-Turkey Recep Tayyip Erdoğan Training and Research Hospital, Department of Public Health – Mogadishu, Somalia.
*Corresponding author: dresrakeles@hotmail.com
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Many studies have been conducted on vitamin D in different age groups, diseases, ethnic groups, and populations. However, our knowledge on vitamin D levels among people in Somalia is limited. To be the baseline for future research, to the best of our knowledge, this is the first comprehensive study to be reported from Somalia. Therefore, we aimed to examine the prevalence and risk factors of vitamin D deficiency among patients attending a tertiary hospital in Somalia.

METHODS

This retrospective study was carried out by examining the results of serum 25(O.H.) D tests of 28,125 patients who were admitted to Somalia Mogadishu-Turkey Training and Research Hospital between January 2017 and December 2021. Our institution is the largest hospital in Somalia and provides a tertiary level of care to more than 2 million people in Mogadishu. The city of Mogadishu, where the study was conducted, is located at 2°4' north, 45°22' east latitude, with an average daily and annual sunlight of 8.4 and 3066 h, respectively, and receives 70% of possible sunlight.

The data were retrieved from the hospital's electronic database following the approval of the Research Ethics Committee of Somalia Mogadishu-Turkey Recep Tayyip Erdogan Training and Research Hospital (Approval number: 21.09.2021, MSTH/7426). Informed consent from study participants was waived due to the retrospective nature of the study. Abstracted data included age, gender, and year. Repeated measurements obtained from the same patient in different admissions were excluded from the analysis.

The 25(O.H.) D levels were measured by the chemiluminescent immunoassay method with the Mindray CL-2000i Chemiluminescence Immunoassay System in the biochemistry laboratory. According to the Endocrine Society Clinical Practice Guidelines, patients were divided into four categories based on their serum 25(O.H.) D levels: vitamin D sufficiency was defined as >30 ng/mL, insufficiency as 20–30 ng/mL, deficiency as 10–19 ng/mL, and severe deficiency as <10 ng/mL[8,9].

Statistical analysis

Analysis was carried out using Statistical Package for the Social Science (IBM SPSS, Version 25.0. Armonk, NY: IBM Corp.) for the Windows software. Data were expressed as frequency (n) and percentage (%) for qualitative variables and arithmetic mean and standard deviation values (Mean±SD) for quantitative variables. The χ^2 test or Fisher's exact test was used for categorical variables, and the independent-sample t-test was used for continuous variables. The one-way analysis of variance (ANOVA) test followed by the Tukey post-hoc test was performed for multiple comparisons. The type I error rate was set at 0.05. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 28,125 patients were enrolled in the study. The mean age of patients was 44.27±20.4 years, and 59.8% were females. The majority of patients were in the age group of 19–40 years. The mean serum level of 25(O.H.) D was 28.42±15.34 ng/mL. Of the patients included in the study, 5.8% (1,618/28,125) had vitamin D sufficiency, 6.5% (1,826/28,125) had vitamin D insufficiency, 41.8% (11,761/28,125) had vitamin D deficiency, and 45.9% (12,920/28,125) had severe vitamin D deficiency. The baseline characteristics of the patients are shown in Table 1.

Table 2 presents the differences in mean 25(O.H.) D levels between groups stratified by age, gender, and year. When the mean serum 25(O.H.) D levels were evaluated according to the years, it was highest in 2017 and 2021 and was lowest

Table 1. Baseline characteristics of the patients (n=28125).

	n (%)
Year	
2017	5043 (17.9)
2018	5285 (18.8)
2019	5153 (18.3)
2020	5615 (20)
2021	7029 (25)
Gender	
Male	11301 (40.2)
Female	16824 (59.8)
Age groups (years)	
0–12	1491 (5.3)
13–18	1083 (3.9)
19–40	9332 (33.2)
41–60	8530 (30.3)
>60	7689 (27.3)
Age (Mean ± SD)	44.27±20.4
25(O.H.) D (ng/mL)	
<10	12920 (45.9)
10–19	11761 (41.8)
20–30	1826 (6.5)
>30	1618 (5.8)
25(O.H.) D level (Mean ± SD)	28.42±15.34

25(O.H.) D: 25-hydroxy-vitamin D.

in 2018 (p<0.001). The mean serum 25(O.H.) D levels were 33.18±16.74 ng/mL for the males and 25.23±13.4 ng/mL for the females. The mean serum 25(O.H.) D levels were lower in females than in males (p<0.001). The mean serum 25(O.H.) D levels according to age groups were as follows: 37.67 ng/mL for 0–12 years of age, 25.86 ng/mL for 13–18 years of age, 26.17 ng/mL for 19–40 years of age, 27.78 ng/mL for 41–60 years of age, and 30.44 ng/mL for >60 years of age. The mean levels of 25(O.H.) D among children 0–12 years of age were significantly higher than those found for the other age groups (p<0.001).

The prevalence of vitamin D severe deficiency (<10 ng/mL) was lowest in 0–12 years of age (23.8%), while the highest rates of prevalence were noted in females (54.8%), in those 19–40 years of age (51.3%), and 2018 (57.1%). A comparison of sociodemographic characteristics according to the serum 25(O.H.) D level is shown in Table 3.

Table 2. Patient characteristics stratified by mean serum 25-hydroxyvitamin D level.

Characteristics	Mean serum 25-hydroxyvitamin D level	p-value
Year		
2017	30.8±14.98	<0.001*
2018	24.52±12.86	
2019	26.21±13.97	
2020	28.75±14.87	
2021	31.01±17.61	
Gender		
Male	33.18±16.74	<0.001*
Female	25.23±13.4	
Age (years)		
0–12	37.67±18.85	<0.001*
13–18	25.86±14.49	
19–40	26.17±13.48	
41–60	27.78±14.59	
>60	30.44±16.64	

*p<0.05.

DISCUSSION

Vitamin D deficiency, which is becoming a serious health problem worldwide, is an issue that affects many individuals of all ages and genders. The overwhelming frequency of vitamin D deficiency among Somali patients at this largest tertiary hospital in Mogadishu, Somalia, is the major finding of this 5-year retrospective study. As a result, our study found that the prevalence of vitamin status levels varied depending on their thresholds; the highest prevalence was severe vitamin D deficiency, which accounted for 45.9% (12,920/28,125),

Table 3. Comparison of sociodemographic characteristics of the study population according to the serum 25-hydroxyvitamin D level.

25(OH) D level intervals (ng/mL)	<10 ng/mL	10–19 ng/mL	20–30 ng/mL	>30 ng/mL	p-value
Year					
2017	1965 (39)	2580 (51.2)	477 (9.5)	21 (0.4)	<0.001*
2018	3019 (57.1)	2098 (39.7)	141 (2.7)	27 (0.5)	
2019	2792 (54.2)	2108 (40.9)	211 (4.1)	42 (0.8)	
2020	2289 (40.8)	2320 (41.3)	343 (6.1)	663 (11.8)	
2021	2855 (40.6)	2655 (37.8)	654 (9.3)	865 (12.3)	
Gender					
Male	3698 (32.7)	5689 (50.3)	1208 (10.7)	706 (6.2)	<0.001*
Female	9222 (54.8)	6072 (36.1)	618 (3.7)	912 (5.4)	
Age groups (years)					
0–12	355 (23.8)	779 (52.2)	241 (16.2)	116 (7.8)	<0.001*
13–18	580 (53.6)	384 (35.5)	54 (5)	65 (6)	
19–40	4788 (51.3)	3628 (38.9)	389 (4.2)	527 (5.6)	
41–60	4012 (47)	3597 (42.2)	491 (5.8)	430 (5)	
>60	3185 (41.4)	3373 (43.9)	651 (8.5)	480 (6.2)	

*p<0.05.

followed by vitamin D deficiency, which accounted for 41.8% (11,761/28,125). The other vitamin D thresholds had a low prevalence of 5.8% (1,618/28,125) in vitamin D sufficiency and 6.5% (1,826/28,125) in vitamin D insufficiency. In our study, the mean serum level of 25(O.H.) D was 28.42±15.34 ng/mL. A study conducted in Riyadh, Saudi Arabia, showed a mean serum 25(O.H.) D level of 35.5±30.6 ng/mL[10]. Another study in Kathmandu, Nepal, found that the total mean serum vitamin D was 19.69±13.68 ng/mL[11]. The findings of this study showed that vitamin D deficiency among Somali patients attending this largest tertiary care hospital is alarmingly high.

Although Somalia is in one of the sunniest parts of the world, our study has similar vitamin D status problems to some parts of Africa, Asia, and the Middle East[12]. A recent systematic review including 195 studies from 44 countries worldwide which used the same cutoff points in our study presented that 88.1% had a mean of 25(O.H.) D values below 30 ng/mL[13], whereas another recent systematic review and meta-analysis study found that one in five people living in Africa had a low 25(O.H.) D concentration using a less than 30 nmol/L cutoff point[3]. In Saudi Arabia, vitamin D deficiency and insufficiency reached 67.8%[10]. In Benghazi, Libya, the estimated vitamin D deficiency was 76.1.1%, and insufficiency was 15.2%[14]. In Kathmandu, Nepal, vitamin D deficiency was 69.6%, and insufficiency was 16%[11]. In Lebanon, vitamin D deficiency was 63%, and insufficiency was 20.5%[15]. In Egypt, the estimated prevalence was 77% for vitamin D deficiency and 15% for vitamin D insufficiency[16].

According to the gender variation of vitamin D deficiency in this study, the female population (59.8%) was more predominant than the male population (40.2%). Globally, the female gender is one of the most important predictors of vitamin D deficiency[13,14]. The increased frequency seen in females was comparable with other studies in Saudi Arabia (78.1%)[10], Libya (58.8%)[14], Nepal (76.1%)[11], and Bangladesh (46%)[17]. This finding was due to some factors that females predominate for vitamin D deficiency. Females are 2.8 times more likely to develop vitamin D deficiency than males. Other factors, such as cultural factors (clothing styles), reduced outdoor activities, aggressive sun protection, and low vitamin D intake, could contribute to vitamin D deficiency[9,14].

This study found that the mean age was 44.27±20.4 years. The majority of patients were in the age group of 19–40 years. The mean age was 36.2±0.9 years in Benghazi, Libya[14], 40.5±14.4 years in Kathmandu, Nepal[11], 47±16.3 years in Chattogram, Bangladesh[18], and 46.9±16.3 in Riyadh, Saudi Arabia[10].

Our study showed that the mean serum vitamin D concentration among genders was 33.18±16.74 ng/mL in males and 25.23±13.4 ng/mL in females. The mean level of serum vitamin D concentrations was lower in females than in males. In Benghazi, Libya, a study showed that the mean serum vitamin D concentrations by gender were 15.4 ng/mL (95%CI 14.6–16.2) in males and 13.2 ng/mL (95%CI 12.5–13.9) in females[14]. A study similar to our study conducted in Kathmandu, Nepal, showed that the mean serum vitamin D concentration by gender was 22.38±17.07 ng/mL in males and 18.89±15.25 ng/mL in females[11].

Vitamin D deficiency was seen in 54.8% of female patients and 51.3% in the group aged 19–40 years, while the least of the patients with vitamin D levels were found in the group aged 13–18 years. Various studies reported a similar observation of lowering vitamin D status levels among females and older people, such as studies in Saudi Arabia, Libya, and Nepal[10,11,14].

The observation of various reports of older people or increased ages that have lowered vitamin D serum concentration is because the older group might not be having vitamin D and calcium supplements or decreased dietary intake, diminished sunlight exposure, reduced skin thickness, impaired intestinal absorption, and impaired hydroxylation of vitamin D in liver and kidney[15-17]. Another necessary explanation is that those younger ages in this study have higher vitamin D levels than older ages where the MENA region (the Middle East/Africa) generally spends more time outdoors compared with other age groups with lower vitamin D levels[13,14,18]. Vitamin D deficiency is predominant in females due to a diet lack of calcium and vitamin D intake, lack of exposure to sunlight due to indoor lifestyle of females, low education levels, and the whole body covering among Muslim women[19,20].

Limitations

Several limitations of the study need to be acknowledged. First, the study findings were limited by the use of a retrospective design. Thus, it did not represent the whole population. Second, the study could not analyze the related risk factors and variables that are important in vitamin D status. Notwithstanding these limitations, this study represents the first comprehensive assessment of vitamin D levels in Somali people living in Somalia. Additionally, the large sample size and inclusion of all age groups removed selection bias and increased the generalizability of the results.

CONCLUSION

The study highlighted a high prevalence of vitamin D deficiency among patients attending our largest tertiary care hospital, particularly among female and older people. To reduce vitamin D

deficiency, we recommend developing intensive educational and awareness programs and campaigns to increase the population knowledge and limit the spread of vitamin D deficiency that engulfs the nation. Furthermore, a large-scale, multicentric, or community-based study should be conducted in the near future to determine the more accurate prevalence and to assess the factors that contribute to the vitamin D level of different modifiable and non-modifiable factors to the health problem of the hypovitaminosis-D burden.

ETHICAL STATEMENT

Ethical approval for this research was provided by the Somalia Mogadishu-Turkey Recep Tayyip Erdogan Training and Research Hospital Ethics Committee (Approval number: 21.09.2021-MSTH/7426). The database management under privacy legislation and the presented study followed the ethical principle of the Declaration of Helsinki.

AVAILABILITY OF DATA AND MATERIALS

The dataset used and analyzed in the study is available from the corresponding author upon reasonable request.

AUTHORS' CONTRIBUTIONS

MAN: Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **EK:** Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **MAHK:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **HHE:** Project administration, Supervision, Writing – original draft, Writing – review & editing. **MAH:** Investigation, Resources, Validation, Writing – original draft, Writing – review & editing. **KNB:** Formal Analysis, Software, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Audience response system smartphone application as an adjunct to tuberculosis teaching for medical students during the coronavirus disease 2019 pandemic

Vagner Kunz Cabral[1], Otávio Augusto Gonçalves Dias Cionek[2], Marcelle Martinez Loureiro[2], Rosa Elisa Bernardo Simbine[1,3], Denise Rossato Silva[1,2*]

SUMMARY

OBJECTIVE: The growing availability of devices for mobile learning has created new opportunities for teaching. With the development of smartphone apps based on audience response systems, there is a possibility to quickly assess student knowledge. The education of health professionals, including medical students, is an essential strategy for tuberculosis control. In the context of the coronavirus disease 2019 pandemic, audience response systems are very useful as online assessment tools. The aim of this study was to use the audience response systems Socrative to assess medical students during a class on tuberculosis.

METHODS: This is a quasi-experimental before-and-after study, with pre- and post-tests carried out through the Socrative app, respectively, before and after a lecture on tuberculosis for medical students. Also, a cross-sectional study was carried out after the course to evaluate the participant's satisfaction through an electronic, structured questionnaire with a Likert-type scale.

RESULTS: A total of 126 students were included in the study. The overall mean pre- and post-test scores were 5.98±1.59 and 8.37±1.36, respectively, with a statistically significant difference ($p<0.0001$). Almost all students were totally satisfied with the use of Socrative on pre- and post-tests.

CONCLUSION: This study describes how the use of Socrative in a tuberculosis class was well received by students. In addition, the baseline knowledge on tuberculosis was low in some topics, with some improvement after the lecture. These findings emphasize the need to further improve the students' knowledge on tuberculosis and help instructors customize the lecture based on the gaps identified in the Socrative assessment.

KEYWORDS: Tuberculosis. Distance education. Information technology. Distance learning. Health education.

INTRODUCTION

The growing availability of devices for mobile learning (m-learning) has created new opportunities for teaching and assessment. With the development of smartphone apps based on audience response systems (ARSs), there is the possibility to quickly assess student knowledge. ARSs are common tools that teachers can implement in classes, which can identify knowledge gaps, guide teaching, and enhance education. ARSs allow you to collect real-time responses from an audience during a lesson. For this, both hardware and software, such as electronic devices called clickers and applications for smartphones, can be used. Socrative (MasteryConnect, Salt Lake City, UT, USA) is a convenient, free ARS app that can be downloaded to personal handheld devices and used by teachers and students. It can also be accessed directly online at socrative.com. The anonymity and simplicity of the process may be of interest to more introverted students and may possibly reduce participation anxiety[1,2]. In the context of the coronavirus disease 2019 (COVID-19) pandemic, it is very useful as an online assessment tool. In fact, due to the COVID-19 pandemic, teaching practices were very limited, with classes and courses shifted to online platforms[3-5].

The education of health professionals, including medical students, is an essential strategy for the control of tuberculosis (TB), enabling the early detection and adequate treatment of TB cases[6]. During the COVID-19 pandemic, TB teaching was impaired, and alternatives had to be sought. No studies to date have evaluated the use of an online assessment tool to evaluate medical students' knowledge of TB. Therefore, the aim of this study is to use Socrative to assess medical students during a class on TB during the COVID-19 pandemic.

[1]Universidade Federal do Rio Grande do Sul, Postgraduate Program in Pneumological Sciences – Porto Alegre (RS), Brazil.
[2]Universidade Federal do Rio Grande do Sul, Faculty of Medicine – Porto Alegre (RS), Brazil.
[3]Ministry of Health – Maputo, Mozambique.
*Corresponding author: denise.rossato@terra.com.br
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METHODS

Study design and location

We conducted a quasi-experimental before-and-after study, with pre- and post-tests carried out through the Socrative app, respectively, before and after a lecture on TB. Also, a cross-sectional study was carried out after the course to evaluate the participant's satisfaction through an electronic, structured questionnaire with a Likert-type scale.

Medical students were recruited at the Hospital de Clinicas de Porto Alegre (HCPA), linked to the Faculty of Medicine of the Federal University of Rio Grande do Sul (UFRGS). The study was approved by the Ethics Committee at the Hospital de Clínicas de Porto Alegre (number 20190051). All participants signed an informed consent form prior to inclusion in the study.

Participants

Third-year medical students from the Faculty of Medicine of the Federal University of Rio Grande do Sul were included in the study. During the mandatory internship of these students in pulmonology, they have several lectures in the schedule of activities, including one on TB.

Lecture on tuberculosis and data collection

Organizational and methodological aspects

It was carried out through a lecture of about 30 min, including a pre-test to assess the participant's current knowledge and a post-test with the repetition of the pre-test questions immediately after the class to assess what participants learned in class. The tests contained nine questions. The objectives of the class were to update participants on the main concepts in TB patient care and to guide students about the actions taken with TB patients.

Content

The content to be covered included (1) TB: Concepts and Epidemiology; (2) Pathogenesis and Pathophysiology of TB; (3) Diagnosis of TB; and (4) Treatment of TB.

Technological aspects

To carry out the pre- and post-tests, the Socrative smartphone app (MasteryConnect, Salt Lake City, UT, USA) was used. To answer the questions, students accessed Socrative directly online or via the app.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY, IBM Corp.). Data were presented as the number of cases, mean±standard deviation (SD), or median with interquartile range (IQR). We measured learners' baseline knowledge (pre-test) and knowledge upon completion of the learning modules (post-test). A score for the pre-test and a score for the post-test were calculated. The Wilcoxon test was used to assess if the learners increased their knowledge to a statistically significant degree. To compare the number of correct answers in pre- and post-test questions, we used the chi-square test, using Yates's correction if indicated, or Fisher's exact test. A value of $p<0.05$ was considered statistically significant.

To calculate the sample size, we estimated a percentage of correct answers of 60% (before intervention) and 80% after intervention. Thus, considering a confidence level of 95% and a beta error of 0.20, at least 79 participants should be included.

RESULTS

During the study period (January 2021 to December 2022), 126 students who met the inclusion criteria were invited and accepted to participate in the study. The mean age of students was 20.5±0.7 years, and 73 (57.9%) were females. The overall mean pre- and post-test scores were 5.98±1.59 and 8.37±1.36, respectively, with a statistically significant difference ($p<0.0001$). Table 1 shows the distribution of the frequency of correct answers in the pre- and post-tests.

At baseline, the frequency of correct answers was very low in some questions: BCG vaccine (51.6%), primary TB (35.7%), characteristics of TB in smokers (9.5%), and characteristics of TB pleural effusion (42.9%). The questions on primary TB and characteristics of TB pleural effusion remained with a low percentage of correct answers (57.1 and 48.4%, respectively).

Table 2 shows the distribution of frequencies and percentages regarding the participants' satisfaction with class on TB. The majority of students said that they were satisfied or totally satisfied with methodology (84.1%), class content (88.1%), content update (92.1%), and that learning focuses on subjects of interest (92.1%). Almost all students were totally satisfied with the use of Socrative on pre- and post-tests (97.6%).

DISCUSSION

In the present study, we demonstrated that almost all students were totally satisfied with the use of Socrative in class. In addition, students' baseline knowledge was low regarding the BCG vaccine, primary TB, characteristics of TB in smokers, and characteristics of TB pleural effusion. After a class on TB, a significant improvement in knowledge was detected,

Table 1. Distribution of frequency of correct answers in pre- and post-tests.

	Pre-test n (%)	Post-test n (%)	p-value
Association between TB and HIV	95 (75.4)	107 (84.9)	<0.0001
Characteristics of LTBI	96 (76.2)	108 (85.7)	<0.0001
BCG vaccine	65 (51.6)	96 (76.2)	<0.0001
Primary TB	45 (35.7)	72 (57.1)	<0.0001
Post-primary TB	95 (75.4)	105 (83.3)	0.001
Association between smoking and TB (increased risk of TB infection and death)	92 (73.0)	115 (91.3)	<0.0001
Characteristics of TB in smokers	12 (9.5)	93 (73.8)	<0.0001
Characteristics of TB pleural effusion	54 (42.9)	61 (48.4)	0.114
TB diagnostic tests	96 (76.2)	106 (84.1)	0.001

TB: tuberculosis; HIV: human immunodeficiency virus; LTBI: latent tuberculosis infection; BCG: bacillus Calmette-Guérin.

Table 2. Participants' satisfaction with class on tuberculosis.

	Totally dissatisfied (%)	Dissatisfied (%)	Satisfied (%)	Totally satisfied (%)
Methodology	0 (0)	1 (0.8)	19 (15.1)	106 (84.1)
Class content: relevance, suitability of content, and organization	0 (0)	0 (0)	15 (11.9)	111 (88.1)
Use of Socrative in pre- and post-tests	0 (0)	0 (0)	3 (2.4)	123 (97.6)
Content update	0 (0)	1 (0.8)	9 (7.1)	116 (92.1)
The learning focuses on subjects of interest	0 (0)	0 (0)	10 (7.9)	116 (92.1)

Data are presented as number (%). TB: tuberculosis.

although some questions remained with a low percentage of correct answers.

Education and training on TB infection and disease are an important part of a TB infection control program[7]. Available evidence suggests that the quality of training has a strong impact on the quality of care provided to patients. A minimum TB training should be standardized and incorporated into medical schools' curricula, especially in setting with medium-high TB burden[8].

During the COVID-19 pandemic, classes were online and, given the impossibility of conducting face-to-face assessments, online assessment tools became especially useful. In this context, Socrative stimulates student engagement during distant online teaching and allows teachers to conduct real-time assessments[3-5]. In addition, instant feedback on correct answers can be given to students, and teachers can identify gaps in knowledge and modify future lectures[9]. This is the case for some questions that remained with a low percentage of correct answers in the present study. Providing feedback helps to establish an interactive learning environment[10].

We showed that almost all students were totally satisfied with the use of Socrative in class. In fact, students found that ARS promotes a better environment for interaction and participation in class[11]. Asking and receiving answers anonymously, without embarrassment, helps them participate more actively in class[2,12,13].

Utilization of ARS during lectures has been shown to enhance learning and increase exam performance[14,15]. A prospective, randomized controlled trial compared one group of residents who received ARS lectures with the other group who received traditional lectures. The authors found that there was 21% improvement between pre- and post-test scores in the ARS lecture group and 2% improvement in the traditional lecture group (p=0.018)[16]. In another randomized trial, radiology residents who used ARS integrated into the lecture had significantly higher knowledge in a test performed on the day of the lecture and long-term retention 3 months later[17].

This study has some methodological limitations. First, the study was conducted in a single center; however, we believe that these results may apply to other settings. Second, we did not include a control group, so we cannot presume that the changes in participants' knowledge were attributable only to TB class and the use of Socrative. Finally, we did not formally assess the validity and reliability of the questions, since this requires a considerable number of stages and is time-consuming and costly, and it is possible that we would not have been able to carry out the study at the height of the pandemic.

Despite these limitations, the findings of the present study highlight the importance of mobile technology and ARS in promoting more interactive lectures.

In conclusion, this study describes how the use of Socrative in a TB class was well received by students. In addition, the baseline knowledge on TB was low in some topics, with some improvement after the lecture. These findings emphasize the need to further improve the students' knowledge on TB and help instructors customize the lecture based on the gaps identified in the Socrative assessment.

AUTHORS' CONTRIBUTIONS

VKC: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original draft. **OAGDC:** Conceptualization, Investigation, Methodology, Writing – review & editing. **MML:** Conceptualization, Investigation, Methodology, Writing – review & editing. **REBS:** Conceptualization, Investigation, Methodology, Writing – review & editing. DRS: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Writing – original draft.

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REVIEW ARTICLE

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Sleep deprivation induces genetic damage in mammalian cells: a systematic review

Daniel Vitor de Souza[1], Barbara dos Anjos Rosario[1], Milena de Barros Viana[1], Luciana Pellegrini Pisani[1], Glenda Nicioli da Silva[2], Daniel Araki Ribeiro[1]*

INTRODUCTION

Sleep is a natural biological state for reducing wakefulness, metabolism, and motor activity characterized by a reversible state and lack of responsiveness to some stimuli[1,2]. According to the American Academy of Sleep Medicine, the phenomenon can be classified into two stages: non-rapid eye movement (NREM – N1, N2, and N3) sleep stages and rapid eye movement (REM) sleep (R) stage[3].

Sleep has also been associated with functional brain connectivity and is required for processing information, energy conservation, and restoration[4]. Sleep deprivation occurs when an individual does not sleep well or even insufficient quantity or low quality of sleep, which leads to a decreasing performance and subsequent deterioration in general health[5]. This condition can impair several behavioral and biological activities, affecting cognition and mood, increasing fatigue, and decreasing vigor. This picture impairs speed, decision-making, and accuracy of motor tasks[6].

Although some environmental factors can interfere with the duration as well as the quality of sleep, it is also genetically controlled[7]. In particular, some studies have demonstrated that sleep deficiency leads to the injury to deoxyribonucleic acid (DNA) in mammalian cells, leading to cellular injury[8-10]. This is consistent with the idea that sleep loss could induce genotoxicity[11]. As a result, this systematic review was motivated to answer the following question: Can sleep deprivation induce genetic damage in mammalian cells?

METHODS

Search strategy

In this research, we evaluated genetic damage in mammalian cells induced by sleep deficiency. This systematic review was performed according to the methodology described in the PRISMA guidelines statement[12]. For this purpose, a search was performed on the following scientific databases: PubMed/Medline, Scopus, and Web of Science, and all studies published in the past 10 years (2013–2023) that investigated the relationship between genetic damage and sleep loss were searched. All articles using a combination of the following keywords were selected: "sleep deprivation," "sleep loss," "paradoxical sleep deprivation," "genotoxicity," "genetic damage," "DNA damage," "comet assay," "single-cell gel electrophoresis," "mutation," "sister chromatid exchange," and "micronucleus assay" to refine the search strategy. Boolean operators were used (AND and OR) to combine the descriptors through different combinations as described elsewhere[13].

Data extraction

The following data were presented using a particular data collection form: year of study, study design, origin, number of individuals, genotoxicity assay, species used, methodological parameters, negative and positive control groups, blind analysis and statistics, main results, and conclusion.

Risk of bias in individual studies

The quality assessment of the selected articles was based on previous studies published elsewhere[13]. The following information from the quality instrument was used: (1) study design, (2) identification and treatment of confounding factors, (3) blind analysis, and (4) data analysis. The criteria used to evaluate the study design were the number of participants per group, statistical analysis, and blind analysis. The confounding factors considered were cytotoxicity, number of repetitions, and positive and negative controls. After that, strong, moderate,

[1]Universidade Federal de São Paulo, Institute of Health and Society, Department of Biosciences – Santos (SP), Brazil.
[2]Universidade Federal de Ouro Preto, Laboratory of Clinical Research – Ouro Preto (MG), Brazil.
*Corresponding author: daribeiro@unifesp.br
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and weak classifications were used as follows: the study was considered strong when it showed dominance on all items, except one; if it was on two items, it was considered moderate; and if the study did not control three or more items, it was considered weak.

RESULTS

Study selection

Initially, the study was able to identify 279 papers, of which 189 publications that were duplicates were excluded from the analysis. After screening all the articles, 161 studies that were not relevant were removed. In addition, reviews, case reports, editorials, papers not written in English, or letters to the editor were not considered. Finally, full texts of the remaining eight studies were sought and thoroughly read by two authors (DVS and DAR). The search strategy is demonstrated in Figure 1.

Variables related to sleep deprivation and genotoxicity (confounders)

All variables evaluated in the studies are demonstrated in Table 1. The studies evaluated DNA damage by different methodologies. Alkaline single-cell gel (comet) assay was performed in three studies[8-10]. TUNEL assay was applied by Everson et al.[11], counting cells into slides. Plasma or urine levels of 8-OHdG were evaluated by Everson et al.[11], Valvassori et al.[12], and Zou et al.[14]. Zhang et al.[13] performed FISH using telomere length as a numerical parameter of genotoxicity.

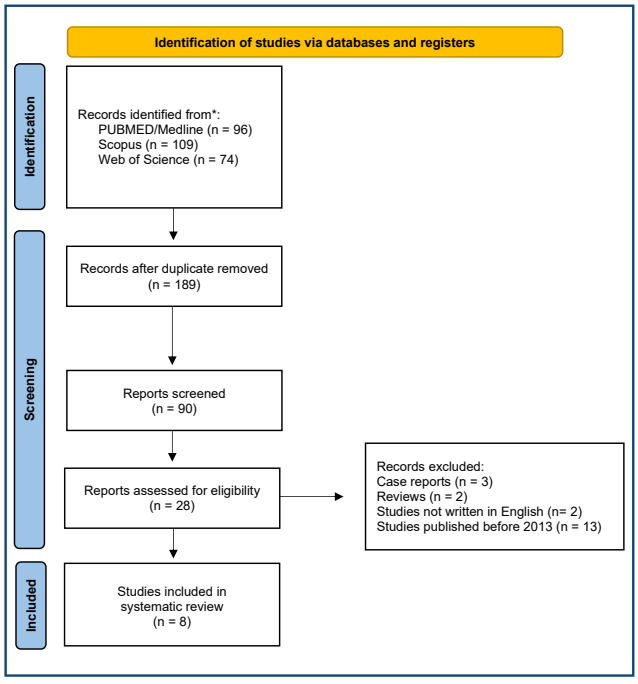

Figure 1. Flowchart of the study.

Main results

In the study conducted by Tenorio et al.[9], the genotoxic effect was seen in the peripheral blood, liver, heart, and brain cells of obese old rats submitted to sleep deprivation.

Regarding oxidative DNA damage, 8-OHdG expression was increased in the liver, jejunum, and lung of rats exposed to total sleep deprivation[11]. Similarly, brain cells increased 8-OHdG in mice exposed to paradoxical sleep deprivation[12]. In humans, the same result was observed in urine samples[14].

The study conducted by Cheung et al.[10] showed an increase in DNA strand breaks in peripheral blood cells of humans after sleep deprivation. In the study conducted by Zhang et al.[13], sleep deprivation was associated with telomere shortening in the bone marrow and testis cells of mice and in the peripheral blood cells of humans. Conversely, the studies conducted by Kahan et al.[8] and Moreno-Villanueva et al.[15] did not show positive genotoxicity in the blood cells of sleep-deprived humans.

Assessment of the risk of bias

The quality assessment of manuscripts is shown in Table 2. After reviewing all studies, five papers were classified as strong[8,9,14,15]. In addition, two studies were categorized as moderate at the final rating, because they did not control two relevant variables[11,13]. Finally, two studies were categorized as weak[10,12].

DISCUSSION

The aim of this study was to evaluate if, and to what extent, sleep deprivation induces genetic injuries in mammalian cells. For this purpose, a total of eight studies were selected in this setting. The single-cell (comet) gel assay is an excellent, reliable method for evaluating DNA strand breakage, including DNA adducts, single- and double-strand breaks, and deficient repair sites. This technique is a simple method that allows the proper investigation of DNA strand breaks that can originate from many contexts and paradigms[16]. In this review, the comet assay was the preferred method for evaluating genetic damage by sleep deprivation as the majority of papers (three studies) have demonstrated positive genotoxicity induced by sleep deprivation in multiple organs of rodents by comet assay. In fact, it has been assumed that DNA damage is driven by sleep[17]. This is because sleep induces nuclear stability, i.e., sleep regulates the homeostatic balance between genetic damage and DNA repair system[17]. Nevertheless, it remains obscure how DNA damage is induced by sleep, and the role of the DNA repair system in

Table 1. Variables analyzed in the studies in chronological order.

Author	Target organs	n	Negative control	Positive control	Assay	Number of cells evaluated	Cytotoxicity	Evaluated parameters	Blind analysis	Proper statistics description	Experimental design associated with other conditions
Cheung et al.[10]	Peripheral blood	24 volunteers 9 Males 15 Females 20.08±2.42 years of age	Yes	No	Alkaline comet	100 comets	No	DNA damage %	No	Yes	–
Everson et al.[11]	Liver Lung Heart Jejunum Spleen	Control rats (n=7) Sleep deprivation (n=7–11) Recovery (n=5–6)	Yes	No	TUNEL 8-OHdG	Four sections –	Yes	Counting cells pg 8OHdG/μg DNA	No	Yes	–
Kahan et al.[8]	Skin	12 mice (n=4/group)	Yes	Yes	Alkaline comet	50 comets	No	Tail intensity and tail moment	Yes	Yes	Aging
Moreno-Villanueva et al.[15]	Peripheral blood	16 volunteers 8 Males 7 Females 36.4 ± 7.1 years of age	Yes	Yes	FADU	–	Yes	DNA intensity	No	Yes	Radiation ex vivo
Tenorio et al.[9]	Peripheral blood Heart Kidney Liver Brain	60 rats (n=25/group)	Yes	Yes	Alkaline comet	50 comets	No	Tail intensity	Yes	Yes	Obesity and aging
Valvassori et al.[12]	Brain	40 mice (n=10/group)	Yes	No	8-OHdG	–	No	Plasma concentration	No	Yes	Lithium
Zhang et al.[13]	Lymphocytes Bone marrow Testis	96 volunteers 28 mice (n=7/group)	Yes	Yes	FISH	–	No	Telomere length	No	Yes	Folic acid diet
Zou et al.[14]	Urine samples	16 volunteers	Yes	No	8-OHdG	–	No	Plasma concentration	No	Yes	–

SD: sleep deprivation; FADU: fluorometric analysis of DNA unwinding; FISH: fluorescence ***in situ*** hybridization; SCE: sister-chromatid exchange; Dash (–): not applicable.

Table 2. Quality assessment and final rating of the studies in chronological order.

Author	Number of confounders	Details	Final rating
Cheung et al.[10]	3	Positive control; cytotoxicity; and blind analysis	Weak
Everson et al.[11]	2	Positive control and blind analysis	Moderate
Kahan et al.[8]	1	Cytotoxicity	Strong
Moreno-Villanueva et al.[15]	1	Blind analysis	Strong
Tenorio et al.[9]	1	Cytotoxicity	Strong
Valvassori et al.[12]	3	Positive control; cytotoxicity; and blind analysis	Weak
Zhang et al.[13]	2	Cytotoxicity and blind analysis	Moderate
Zou et al.[14]	1	Cytotoxicity	Strong

this scenario. Anyway, these findings suggest that genetic damage plays an important role as a biological regulator of sleep in mammalian cells[18]. In the past decades, the single-cell gel comet assay Expert Group has established some guidelines for conducting the methodology in a proper way[19]. First, it is mandatory to evaluate at least 25 comets per slide. Additionally, the percentage of the tail (known as tail intensity or % DNA in tail) is the best option when analyzing comet assay associated with an image analysis system.

Furthermore, several studies have demonstrated that sleep deprivation can cause DNA damage using other assays, such as FADU and TUNEL tests. Of particular importance, the studies conducted by Everson et al.[11] and Valvassori et al.[12] have demonstrated that sleep deprivation was able to induce oxidative DNA damage, as depicted by 8-OHdG expression. It is important to highlight that 8-OHdG is synthesized from the reaction of the hydroxyl radical (HO•) and guanine, which is the most common way for DNA damage. As a result, a pro-mutagenic agent has been formed when the DNA damage is not repaired[20].

One important reason that can be categorized as a confounding factor in genotoxicity studies is the adoption of negative and positive controls in the experimental design. For any *in vivo* genotoxicity assay, it is mandatory to demonstrate the specificity as well as the sensitivity of the methodology. Most of the studies included in this review performed tests with positive and negative controls. Nevertheless, the studies conducted by Cheung et al.[10] and Everson et al.[11] did not provide concurrent positive control in the experimental design. Another question refers to cytotoxicity. High cytotoxicity is the main confounding factor in genotoxic investigations[21]. Underestimating cytotoxicity may lead to incorrect or misleading data interpretation. In this sense, it is necessary to have more information regarding the association between cytotoxic and genotoxic effects to achieve more sensitive results. Ten studies included in this review did not perform complementary analysis for cytotoxicity.

Considering various parameters used for evaluating the studies included in the review, there is some tendency in the literature showing genotoxic effects that are induced by sleep deprivation. Anyway, such information will bring new insights for a better understanding of the consequences induced by sleep deficiency on genetic material.

AUTHORS' CONTRIBUTIONS

DVS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **BAR:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **DAR:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – original draft, Writing – review & editing. **MBV:** Formal Analysis, Writing – original draft, Writing – review & editing. **LPP:** Formal Analysis, Writing – original draft, Writing – review & editing. **GNS:** Formal analysis, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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Health 4.0 in the medical sector: a narrative review

Antônio Cruz Júnior[1], Eduardo Mário Dias[2], Maria Lídia Dias Scoton[3], Braulio Henrique Magnani Branco[4*]

INTRODUCTION

The introduction of technological resources in health and medicine has led to numerous innovations[1,2]. However, there is still a lack of feedback and decisive actions to deliver services and products that reduce costs and improve evaluation, diagnosis, and medical treatment[3,4]. "Health 4.0" is a concept that encompasses the use of technologies such as Big Data, Internet of Things (IoT), cloud computing, and artificial intelligence (AI) to improve health care. These technologies can potentially improve the quality of care, reduce costs, and make health care more accessible[5].

However, challenges must still be overcome before "Health 4.0" can be fully realized[5-8]. These challenges include the promotion of health literacy, adherence to the use of technologies, and organizing infrastructure for optimal and real-time conduction of the indicators[9-11]. This study aimed to discuss the challenges and possibilities of "Health 4.0" in the medical sector regarding information and knowledge management, efficiency and effectiveness of the service, and the current level of evidence.

METHODS

The SANRA guideline[12] was used to organize this narrative review. Articles indexed in the following databases were used: Latin American and Caribbean Literature in Health Sciences (LILACS), Scientific Electronic Library Online (SciELO), PubMed, and Web of Knowledge, with the following filters: (1) research published in the past 10 years (October 2012 to October 2022) and (2) systematic reviews and/or meta-analyses. The following indexing terms or descriptors in Portuguese and English were used: "health 4.0" and "big data" or "internet of things" or "cloud computing" or "artificial intelligence." A total of 23 articles on the proposed theme were included in the final analyses.

RESULTS AND DISCUSSION

The results of this study are divided into three sessions, including "Health 4.0": (1) information and knowledge management; (2) efficiency and effectiveness of care; and (3) current level of evidence.

Information and knowledge management in "Health 4.0"

The use of devices in 4.0 health has generated data that need to be analyzed to turn it into information that guides evidence-based practice[13]. Medical companies and professionals must establish strategies to manage these data and create mechanisms to explore the information collected[14]. One strategy is to use software that provides health professionals with specific guidelines or recommendations to assist in their diagnosis, disease management, and treatment.

This software, called Medical Decision Support Systems, can reduce diagnostic time and improve the quality of care for patients[15]. Wearable devices that feature Internet-based technologies, which have been related to monitoring the level of stress, amount, and quality of sleep, asthma, chronic obstructive pulmonary disease, cardiovascular diseases, diabetes and nutrition, aspects related to gait and falls, neurological diseases, recognition of physical activity, and rehabilitation, among other functions[16].

The data processing systems that reproduce human cognitive functions' speed and ability to relate and analyze information exponentially have been discussed in the literature before the economic impacts on health care[17]. Cozzoli et al.[14] discussed

[1]Universidade de Sao Paulo, Institute of Radiology, Clinical Hospital, Faculty of Medicine – São Paulo (SP), Brazil.
[2]Universidade de Sao Paulo, Polytechnic School, Department of Electrical Energy Engineering and Automation – São Paulo (SP), Brazil.
[3]Universidade de Sao Paulo, Electrical Automation in Industrial Systems Group – São Paulo (SP), Brazil.
[4]Universidade Cesumar, Health Promotion Department – Maringá (PR), Brazil.
*Corresponding author: brauliohmagnani@gmail.com
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that big data analyses are considered a milestone for managing studies applied to health organizations, although scientific research lacks investigations regarding the standardization and integration of devices.

The increase in the potential for big data is associated with continuing medical education, based on (1) transformation of data related to learning with medical systems; (2) intelligence in health based on learning about innovation in health and forecasting processes; (3) data collection to understand the patient's profile; and (4) learning based on clinical decision-making in health[18]. The collected data can boost learning and revolutionize the medical industry since they store up-to-date knowledge from innovative research[18]. Medical companies that acquire medical technologies, hardware, and software must also invest in continuing education and research to make informed decisions about diagnoses, treatments, medication selection, and follow-up[19].

The conduction of randomized clinical trials is fundamental for advancing processes related to Health 4.0, such as the development of artificial technologies, big data, cloud, cybersecurity, telemedicine, and wearable devices, to improve global digital health strategies[20]. It is concluded that processes linked to Health 4.0 need to be tested on a large scale in health centers to improve the systems and the services that will be provided.

Efficiency and effectiveness of care

The literature has discussed the economic evaluation, impact of technologies, and process management of Health 4.0. Voets et al.[21] noted that the economic evaluation of AI is limited to financial costs, and there is a lack of short-, medium-, and long-term evaluations of possible impacts on health. Pinto de Paula Filho and Lamy[22] pointed out that there will be no real progress in the development of Health 4.0 if medical companies do not understand the impacts of these technologies on companies and patient care.

The current level of evidence of "Health 4.0"

The DXplain software is used to compile medical information to make possible diagnoses from laboratory data, history, and symptoms, generating a list in descending order of importance also indicating further investigations, and the HELP system, which is an integrated performance system with a computerized medical record system, which contains patient information[23]. As the doctor enters patient data, the system can make reminders and alerts, interpret data, and diagnose diseases[23].

DXplain has a knowledge base that includes more than 2,400 diseases and more than 5,000 clinical findings in medicine[15]. The PathOS software was developed to support rapid clinical diagnosis needs; this software has proven robust after 2 years of use at the Peter MacCallum Cancer Center for analysis, genetic test reporting, and curation for cancer patients[24]. Esteva et al.[25], using a set of 14,000 images already diagnosed by dermatologists, asked the system to recognize three types of lesions: benign, malignant, and noncancerous growths. The percentage of correct answers for the AI system was 72%, and for dermatologists, it was 66%[25].

The technology can be applied in other specialties if the image is adapted[24]. Bhalodiya, Keung, and Arvanitis[26] observed promising results of AI in identifying tumors via magnetic resonance. Nonetheless, the authors suggest that the algorithms must be improved. Similar responses were identified by Li et al.[27], who argued that AI algorithms require improvements to diagnose non-alcoholic fatty liver disease more assertively. Other studies have investigated highly relevant aspects of the health and quality of life of asthmatics, such as Li et al.[28] who used a sensor to measure airborne formaldehyde levels. However, the study systematized the prototype, but so far, the next step has not yet been carried out, which will be useful to test the equipment's efficiency in monitoring formaldehyde.

In turn, Tran, Ngo, and Tong et al.[29] developed an application for detecting falls based on machine learning, in which the respective authors consider that the technology can differentiate a fall from some other joint event, such as sitting and jumping. Thus, it is considered that the two technologies presented in the studies[28,29] have great potential but need to be tested in controlled clinical trials. During the COVID-19 pandemic, health technologies enabled the elaboration of remote diagnosis through devices, and the non-drug treatment of obesity and associated comorbidities[9], drug treatment, and medical equipment were delivered to isolated areas[30].

Another contribution was monitoring patients infected by the virus through devices and interconnected networks[30]. Al-Arkee et al.[31] pointed out that applications to increase adherence to drug treatment of cardiovascular diseases seem to be effective, but it was discussed which components would be effectively essential for patients. The same authors mentioned that developing large-scale studies would be relevant for improving applications[31]. The study carried out by Nasajpour et al.[30] to determine the role of technologies such as wearables, smartphone applications, and others that are based on IoT in the tracking and control of COVID-19 and how they act in the three main phases – early diagnosis, quarantine time, and after recovery

– showed that, in all phases, the technology based on the IoT showed good and promising results[30,32].

The same authors consider that fine adjustments should be made as more information about the virus's behavior is collected, as this is the only way to reduce the impacts of this type of disease significantly. Considering the heterogeneity of diagnoses[33] before the dissemination of information, diagnosis, and direction of clinical conduct based on the responses of mobile technologies, more clinical, controlled, and randomized studies demand to be carried out to increase the assertiveness of diagnoses based on new technologies.

Recently, Akhtar et al.[34] argued that new technologies have significantly influenced health services, with the beginning of the electronic medical record, a new era of digital health, and the emerging growth of techniques that aim to implement robotic surgeries and algorithms for machine learning, which can even replace the health professionals in the future. Additionally, Battineni, Hossain, and Chintalapudi[35] also pointed out that the information collected in "biobanks" may predict possible pathological outcomes based on AI, which will probably lead to precision medicine research and guide the population's health services.

FINAL CONSIDERATIONS

Health 4.0 has emerged as a promising field that could revolutionize health care. However, more research is needed to validate the effectiveness of these technologies and develop treatment protocols. Medical companies need to deeply understand the technologies already present in Health 4.0 to optimize their services. Integrating new technologies with professionals in this segment can develop predictive, preventive, personalized, and participatory work.

The information presented in this article is expected to guide future research concerning Health 4.0. In particular, randomized clinical trials with the testing of protocols, use of comparison groups, exponent technologies versus conventional treatment, and gold-standard measurement versus new measurement protocols, among other possibilities, are indispensable.

AUTHORS' CONTRIBUTIONS

ACJ: Conceptualization, Data curation, Investigation, Writing – original draft. **EMD:** Conceptualization, Data curation, Investigation, Writing – original draft. **MLDS:** Conceptualization, Data curation, Investigation, Writing – original draft. **BHMB:** Conceptualization, Data curation, Investigation, Writing – original draft.

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