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Editorial Production

LETTER TO THE EDITOR
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Comment on "The effect of mutation status, pathological features and tumor location on prognosis in patients with colorectal cancer"

Zhisong Chen[1], Zhanshuo Gu[1]*

Dear Editor,

Recently, we read an article "The effect of mutation status, pathological features and tumor location on prognosis in patients with colorectal cancer" that analyzes the prognostic factors of colorectal cancer. According to the clinical follow-up data, the genetic and epigenetic risk factors, such as perineural invasion, stage and grade, gender, age, RAS status, and tumor side, on the prognosis of patients with colorectal cancer were analyzed. This retrospective clinical study is of great significance for guiding clinical treatment, especially as part of a future meta-analysis[1].

However, we have some opinions which we want to discuss with the author: First, the treatment of patients with colorectal cancer during follow-up needs to be clearly described. Patients with colorectal cancer with different stages, pathologies, and molecular types will receive different treatment methods. If the impact of treatment on patient survival had not been excluded, the conclusion of this study would have been obviously controversial. For example, most of the studies listed in this article describe the treatment of the observation group[2].

Second, in retrospective clinical studies, the criteria for admission or exclusion should be clear, and it should be based on widely accepted and latest version of literature or guidelines as much as possible. Some references in this study were obsolescence. One can refer to the recent literature to make the research background, design, and implementation more scientific so as to get the conclusion more convincing[3,4].

AUTHORS' CONTRIBUTIONS

ZC: Writing – original draft, Writing – review & editing.
ZG: Writing – original draft, Writing – review & editing.

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LETTER TO THE EDITOR
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Cancer screening and COVID-19 pandemic

Rujittika Mungmunpuntipantip[1]*, Viroj Wiwanitkit[2,3]

Dear Editor,

We would like to share ideas on article "Dealing with cancer screening in the COVID-19 era." Fagundes et al. concluded that "the decision to keep cancer screening must be discussed and individualized, considering the possibility of new waves of COVID-19"[1]. The importance of primary preventive intervention including cancer screening should not be neglected during the COVID-19 pandemic. The practice guidelines might be different in different settings. The coverage rate and cancer detection rate are interesting parameters to be monitored. In our settings, the rates of some cancer screening might be decreased and some might be increased. For example, self-breast examination is increased due to the promotion of this cancer screening practice. Also, the availability of primary care units is still another important factor for promoting cancer screening activity. In the area with maintaining function, cancer screening rate has not been significantly decreased during the COVID-19 outbreak. Risk of delayed screening and cancer detection is recognized during the crisis and it is necessary to have good public health policies to manage it[2]. Mazidimoradi et al. noted that maintaining the capacity of screening services, lifting of restrictions, and reducing fear in the public are necessary for maintaining successful cancer screening during the COVID-19 pandemic[3].

AUTHORS' CONTRIBUTIONS

RM: Conceptualization, Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **VW:** Conceptualization, Formal Analysis, Supervision, Validation, Visualization, Writing – review & editing.

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POINT OF VIEW
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Big gain, no pain: Thyroid minimally invasive FNA (Thy MIFNA): Proposal of novelty in terminology

Ilker Sengul[1,2]*, Demet Sengul[3]

In the field of thyroidology, image-guided interventional procedures have globally been discerned and incrementally utilized over the past four decades. While ultrasonography (US) and fine-needle aspiration (FNA) serve as a diagnostic cornerstone to rule out malignancy in thyroid lesions, *per se*, remaining the main challenge in their management, to a lesser extent, core needle biopsy has been being currently used as another primary diagnostic tool for evaluating this crucial issue. Nevertheless, the debate is still ongoing, and in particular, indeterminate cytology remains to be a highly controversial issue in thyroid cytopathology, a dynamic discipline, to date[1-3]. To deal with this compelling state and resolve this issue, clinical data, sonographic features, elastographic pattern, or outcome of other imaging/interventional techniques should be considered together and also can be supported by the relevant molecular testing. Roles of sonography and FNA are well-established and well-accepted in the diagnosis of thyroid nodules worldwide. However, to the best of our knowledge, the ideal needle size for US-guided FNA (henceforward, FNA) procedure has not been declared in a released well-accepted management guideline, up to now. On the contrary, a wide range of needle sizes, 20–27 gauge (G), have been used for application in the different geographic regions (e.g., 25–27-G in most Western countries and 21–22-G in Japan)[4]. Adequacy of the finer and thicker needles has been reported as similar by many authors[5]. Hanbidge et al.[6] proclaimed no significance between the 23- and 27-G needles in the adequacy of the samples obtained. Of note, the authors asserted that the diagnostic quality of the aspirate may be preferable to the finer one, harboring 27-G[6]. Zhang et al.[7] stated that no statistically significant differences were present between 23-, 25-, and 27-G needles with the adequacy rates (88.5, 90.4, and 89.7%, respectively; p>0.05), involving higher numerical rates in 27-G needle than the 23-G one. However, Tanaka et al.[8] reported that the nondiagnostic/unsatisfactory rates of 22- and 25-G needles were 18.5 and 21.0%, respectively. We have utilized the 27-G needles for our interventional US techniques with surgeon-performed US (SUS) for 10 years, which have been performed by one endocrine surgery sonographer (I.S.), with the nondiagnostic cytology (Category I, The Bethesda System for Reporting Thyroid Cytopathology [TBSRTC], 1st ed.) rate of 9.0%[3-9]. On the other hand, the size/bore of the fine needle for FNA application might also affect the comfort of the patient. The real adequate and comfortable sampling technique with less painful instruments in the case of interventional procedures might be considered as state-of-the-art. "Bonitas non est pessimis esse meliorem." Herewith, we might propound to opt for a 27-G needle for aspiration purposes as an efficient and comfortable tool of choice. In addition, we have administrated preprocedural local anesthesia to the neck region of the cases before the "SUS-based" FNA during the time frame of 10 years. We even have administrated topical anesthesia before administrating the preprocedural local anesthetic agent during the mentioned decade. Currently, on the basis of the scientific reports in the English-language literature and our experiences, we have presented and kindly propounded a novel term, "minimally invasive FNA" (*MIFNA*) and "Thyroid minimally invasive FNA" (*Thy MIFNA*)[10,11]. More recently, we suggested opting for Thy MIFNA with 27-G fine needle in FNA while revisiting optimal needle size for thyroid FNA cytology in terms of diagnostic rate and comfort of the patient as different pears in a pod, which has currently been published in Volume 67, *Revista da Associação Médica Brasileira*[12]. We hope *Thy MIFNA*[10-12], involving preprocedural topical and local anesthesia with 27-G genuine fine needle, to contribute considerably

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in the field of neck-endocrine and endocrine surgery, endocrinology, endocrine pathology, interventional radiology, head & neck surgery, otorhinolaryngology, and thyroidology as a delicate[10-12] and crucial diagnostic tool with a novel terminology.

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AUTHORS' CONTRIBUTIONS

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

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SHORT COMMUNICATION
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Review of the current literature regarding cardiac adverse events following COVID-19 vaccination

Tufan Çınar[1]*, Mert Ilker Hayıroçlu[2], Vedat Çiçek[1], Murat Selçuk[1], Samet Yavuz[1], Ahmet Lütfullah Orhan[1]

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was responsible for coronavirus disease 2019 (COVID-19) infection, was discovered in Wuhan, China, in December 2019[1]. Since then, the disease has spread globally, resulting in a pandemic. Because there is no specific antiviral treatment for COVID-19 disease, vaccination seems to appear the most effective vehicle for controlling the infection. Until now, many vaccines have been developed and approved for immediate use by the health authorities. Two types of messenger RNA (mRNA)-based COVID-19 vaccines, namely, BNT162b2 mRNA (Pfizer-BioNTech, NY) and mRNA-1273 (Moderna, Cambridge, MA), have been administered in hundreds of millions of doses since they have received provisional Food and Drug Administration (FDA) approval in the United States in December 2020[1]. Janssen Ad26.COV2.S (Johnson and Johnson, New Brunswick, NJ) and The ChAdOx1 [Oxford/AstraZeneca (AZD1222)] were recombinant types of vaccines, in which replication-deficient human adenovirus type 26 vector was used to transfer the virus[1]. Although side effects from these vaccines are generally mild and transient, there has been an upsurge of cases with cardiac adverse events reported after COVID-19 vaccination. As a result, the objective of this review was to assess all cardiovascular adverse events reported following COVID-19 immunization, as well as the likely mechanisms behind them.

METHODS

We searched the database of PubMed, Embase, and Cochrane for all possible cardiac adverse events reported after COVID-19 vaccination using the following search inputs until September 13, 2021: "COVID-19 vaccine-induced acute myocarditis," "COVID-19 vaccine-induced acute perimyocarditis," "COVID-19 vaccine-induced acute myocardial infarction," "COVID-19 vaccine-induced ST elevation myocardial infarction," and "COVID-19 vaccine-induced acute coronary syndrome." Only papers written in English were included in this review. Additionally, following a review of the references in the relevant publications, any further papers were collected. Our review was restricted to only cardiac adverse events reported after COVID-19 vaccination. In total, 68 relevant cases were found in the literature. Of them, 61 cases were diagnosed with acute myocarditis (AM), one case with acute perimyocarditis, five cases with acute myocardial infarction (AMI), and one case with Kounis syndrome after COVID-19 vaccination.

Vaccination types

Table 1 describes the vaccine types, symptoms onset, and COVID-19 polymerase chain reaction (PCR) positivity of all published cases. The majority of AM patients who suffered cardiac adverse events after receiving COVID-19 vaccination had previously been immunized with mRNA-based vaccines. [In total = 65 cases, 35 of them with BNT 162b2 (Pfizer) and 30 of them with mRNA-1273 SARS-CoV-2 (Moderna)[2-15].] Only three cases had a history of vaccination with adenovirus vector origin [two with Covishield (AZD1222) and one with Janssen Ad.26.COV2.S (Johnson and Johnson)[2].] Almost all of the AM cases (60/61) were diagnosed following the injection of vaccinations made with mRNA technology, and the majority of them developed

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Table 1. Vaccine types, symptoms onset, and COVID-19 PCR positivity of all published cases.

	Vaccine types	Presentation after the second dose vaccine (%)	History of COVID-19? (%)	Is the patient COVID-19 polymerase chain reaction positive?	Does the patient have a nucleocapsid antibody?	Time between last vaccination and symptom onset, days
Case series[2]	5 BNT162b2 (Pfizer); 1 mRNA-1273 (Moderna); 1 J&J	71	14	6/7 patients were tested, all were negative	4/7 patients tested, all were negative	3 (2–7)
Case series[3]	5 BNT 162b2 (Pfizer); 3 mRNA1273 (Moderna)	88	25	All were negative	NA	3 (1–4)
Case series[4]	BNT 162b2 (Pfizer)	83	No	All were negative	All were negative	2.5 (1–16)
Case series[5]	2 BNT 162b2 (Pfizer), 2 mRNA1273 (Moderna)	100	No	Negative	NA	2.5 (1–5)
Case series[6]	7 BNT 162b2 (Pfizer), 16 mRNA1273 (Moderna)	87	13	19/23 were tested, all were negative	NA	2 (1–4)
Case[7]	BNT62b2 (Pfizer)	100	100	Negative	%100	3
Case[8]	BNT162b2 (Pfizer)	100	No	Negative	Negative	1
Case[9]	BNT162b2 (Pfizer)	100	No	Negative	Negative	3
Case[10]	mRNA-1273 (Moderna)	100	No	Negative	NA	4
Case[11]	mRNA-1273 (Moderna)	100	No	Negative	Negative	1
Case[12]	BNT62b2 (Pfizer)	100	No	Negative	Negative	1
Case[13]	BNT62b2 (Pfizer)	100	No	Negative	Negative	3
Case[14]	mRNA-1273 (Moderna)	100	No	Negative	Negative	1
Case series[15]	BNT62b2 (Pfizer)	100	No	Negative	Negative	12 h–3
Case[16]	BNT62b2 (Pfizer)	No*	No	Negative	Negative	1 h
Case[17]	Covishield (Azd1222)	No*	No	Negative	Negative	2
Case[18]	mRNA-1273 (Moderna)	No*	No	Negative	Negative	1
Case series[19]	mRNA-1273 (Moderna)	No*	No	Negative	Negative	1–5
Case[20]	Azd1222 (Oxford University and AstraZeneca)	No*	No	Negative	Negative	2 h

*Presentation after the first dose vaccine. NA: not applicable.

symptoms 1–3 days after the second dose of immunization (57/61)[2-15]. In contrast to AM cases, the majority of AMI cases (4/5) emerged after the first dose of mRNA-based vaccination was administered[16-19]. Only one case of Kounis syndrome had been reported in the literature, and this allergic response occurred 2 h after the first dose of Covishield (AZD1222) vaccination[20]. Interestingly, the COVID-19 PCR test was negative in all cases.

Baseline clinical characteristics, electrocardiographic findings, and laboratory findings

Table 2 summarizes the baseline characteristics, presenting symptoms, electrocardiography, and laboratory results in all published cases. Patients who were diagnosed with AM were relatively younger and almost all of them were male. By contrast, AMI cases were older. The common complaint in all patients was chest pain. Electrocardiography findings in AM cases ranged from no ischemic changes to ST elevation, PR depression, and nonspecific ST changes[2-15]. Remarkably, patients who presented with AMI following immunization had ST elevation only in inferior leads[16-20]. Troponin levels were measured in all patients who developed a cardiac event after vaccination. In all of them, it was reported above the reference range. The data on brain natriuretic peptide (BNP) levels were shared in very few cases[2,5,10-12,13-15]. On the other hand, C-reactive protein (CRP) levels were elevated in all reported cases. Contrary to COVID-19 infection, lymphopenia was not detected in most patients with post-vaccine cardiac events.

Imaging findings

Table 3 displays the imaging data, in-hospital treatment, and outcomes of all cases. Echocardiography was performed in most cases since it was in the diagnostic algorithm of diseases such as AM and AMI. Left ventricle wall motion defect was observed in all patients with AMI, whereas AM patients had findings in the spectrum from preserved left ventricular ejection fraction (LVEF) without segmental abnormalities to global hypokinesia and low LVEF[2-6,8-11,13-16]. Cardiac magnetic resonance imaging was performed in almost all AM cases, which demonstrated a subepicardial late gadolinium enhancement and myocardial edema compatible with AM[2-15]. Although it was considered the gold standard for the diagnosis of AM, the endomyocardial biopsy was not performed on any patients.

Table 2. Baseline characteristics, presenting symptoms, electrocardiography, and laboratory findings of all published cases.

	Age, gender	Presenting symptoms	Diagnosis	Electrocardiographic (ECG) findings	Lab findings
Case series[2]	24 (19–30), all cases were male	Chest pain was present in all cases, 42% had nonspecific symptoms	AM	4 patients had ST elevations, 1 patient had nonspecific ST/T changes	Lymphopenia: – CRP: elevated in 71% Troponin: elevated in all cases BNP: elevated in 50%
Case series[3]	29 (21–56), all cases were male	Chest pain was present in all cases, 63% had nonspecific symptoms	AM	6 patients had ST elevation, 1 patient had peaked T waves, one patient had normal ECG	Lymphopenia: – CRP: elevated in 88% Troponin: elevated in all cases
Case series[4]	22 (16–45), all cases were male	Chest pain was present in all cases, 33% had nonspecific symptoms	AM	All cases had ST elevations	Lymphopenia: – CRP: elevated in all cases Troponin: elevated in all cases
Case series[5]	30 (23–70), 75% of cases were male	Chest pain was present in all cases, 33% had nonspecific symptoms	AM	All cases had ST elevation, two cases had PR depression	Lymphopenia: – CRP: elevated in all cases Troponin: elevated in all cases BNP: elevated in 50%
Case series[6]	25 (20–51), all cases were male	Chest pain was present in all cases	AM	19/23 cases had ST elevations, T-wave inversions, and nonspecific ST changes	Lymphopenia: – CRP: NA Troponin: elevated in all cases
Case[7]	56, Male	Chest pain	AM	ST elevation	Lymphopenia: – CRP: elevated Troponin: elevated

Continue...

Table 2. Continuation.

	Age, gender	Presenting symptoms	Diagnosis	Electrocardiographic (ECG) findings	Lab findings
Case[8]	39, Male	Chest pain, myalgia, fatigue, fever	AM	ST elevation	Lymphopenia: – CRP: NA Troponin: elevated
Case[9]	30, Male	Chest pain, myalgia, fatigue, fever	AM	ST elevation	Lymphopenia: – CRP: elevated Troponin: elevated
Case[10]	24, Male	Chest pain, myalgia, fatigue, fever	AM	No ischemic changes	Lymphopenia: – CRP: elevated Troponin: elevated BNP: normal
Case[11]	52, Male	Chest pain, myalgia, fatigue, fever	AM	Incomplete right bundle branch block and left axis deviation	Lymphopenia: – CRP: elevated Troponin: elevated BNP: elevated
Case[12]	66, Male	Chest pain, myalgia, fatigue, fever	AM	ST elevation	Lymphopenia: – CRP: NA Troponin: elevated
Case[13]	24, Male	Chest pain, myalgia, fatigue, fever	AM	No ischemic findings	Lymphopenia: – CRP: elevated Troponin: elevated BNP: elevated
Case[14]	34, Male	Chest pain, myalgia, fatigue, fever	AM	Lateral PR depression and ST elevation mirrored in aVR with PR elevation and ST depression	Lymphopenia: – CRP: elevated Troponin: elevated BNP: elevated
Case series[15]	15 and 22, all cases were male	Chest pain, myalgia, fatigue, fever	AM and acute myopericarditis	J-point elevation in the lateral leads with slightly widened QRS complexes and no ischemic findings	Lymphopenia: – CRP: elevated Troponin: elevated BNP: elevated
Case[16]	86, Male	Chest pain, myalgia, fatigue, fever	STEMI	ST elevation inferior wall	Lymphopenia: – CRP: NA Troponin: NA BNP: NA
Case[17]	63, Male	Chest pain, myalgia, fatigue, fever	STEMI	ST elevation in leads II and III and aVF	Lymphopenia: NA CRP: NA Troponin: elevated
Case[18]	96, Female	Chest pain, myalgia, fatigue, fever	STEMI	ST-segment elevation in the anterior and inferior leads	Lymphopenia: NA CRP: NA Troponin: elevated
Case series[19]	42 and 68, 50% of cases were male	Chest pain	STEMI and AMI	ST elevation in leads II and III and aVF	Lymphopenia: NA CRP: NA Troponin: elevated
Case[20]	62, Female	Chest pain	Kounis syndrome	ST elevation in inferior leads (II, III, and AVF) and reciprocal ST segment depression in lead I and AVL	Lymphopenia: NA CRP: NA Troponin: elevated

AM: acute myocarditis; CRP: C-reactive protein; BNP: brain natriuretic peptide; STEMI: ST elevation myocardial infarction; AMI: acute myocardial infarction; NA: not applicable.

Table 3. Imaging findings, in-hospital treatment, and outcomes of all published cases.

	Echocardiographic findings (%)	Cardiac MRI findings	Median hospitalization, days (range)	In-hospital treatment (%)
Case series[2]	Abnormal in 57 [mild hypokinesia in 3, 1 reduced LVEF, one mild LV enlargement], normal in 43.	All cases had LGE, one with wall motion abnormality, three with myocardial edema in T2	3 (2–4)	43 with NSAIDs, 43 with colchicine, 43 with famotidine, 14 with steroids
Case series[3]	All cases had motion abnormality with regional or generalized hypokinesia	All cases had LGE, six with myocardial edema	All cases were reported as stable	38 with NSAIDs, 25 with colchicine, 13 with steroids
Case series[4]	2/6 with hypokinetic segments but preserved LVEF, 4/6 had normal LVEF	All cases had mild subepicardial edema and LGE	6 (4–8)	100 with NSAIDs and colchicine
Case series[5]	One patient with LVEF 40, the others had normal LVEF	All cases had LGE, increased T1 and T2 intensity	3 (2–4)	50 with NSAIDs, 75 with colchicine, 25 with steroid
Case series[6]	4/23 cases had LVEF <50	All cases had subepicardial LGE or focal myocardial edema	NA	NA
Case[7]	NA	LGE and myocardial edema in T2 imaging	7	NA
Case[8]	Normal LVEF	Subepicardial enhancement	6	Anti-inflammatory medications
Case[9]	Abnormal wall motion abnormality and mild pericardial effusion	Subepicardial LGE of the myocardium	7	Beta-blocker, acetylsalicylic acid, steroid
Case[10]	Normal LVEF	Patchy mid-myocardial and epicardial LGE with edema	NA	Beta-blocker
Case[11]	No wall motion abnormalities, LVEF was preserved	Mild myocardial and subepicardial linear and nodular LGE and mild hypokinesia	4	ACE inhibitor, beta-blocker
Case[12]	Reduced LVEF of 44	Edema on T2 sequences and subepicardial enhancement in the lateral mediastinal region	NA	NA
Case[13]	Normal LVEF	Subepicardial enhancement involving the lateral wall	NA	NA
Case[14]	Reduced LVEF of 43 without pericardial effusion	Subepicardial LGE in the anterolateral and inferolateral segments, as well as patchy myocardial edema on T2	5	High-dose aspirin, colchicine, ACE inhibitor, beta-blocker
Case series[15]	Normal LVEF	NA	2	Aspirin, NSAIDs, and colchicine
Case[16]	NA	NA	Not survived	Balloon angioplasty and glycoprotein IIb/IIIa receptor inhibitor (eptifibatide)

Continue...

Table 3. Continuation.

	Echocardiographic findings (%)	Cardiac MRI findings	Median hospitalization, days (range)	In-hospital treatment (%)
Case[17]	Inferior wall hypokinesia with LVEF of 50	NA	5	Thrombolysed with 1.5 million IU streptokinase, and anti-platelets and anti-anginal drugs
Case[18]	Anterior and inferior wall hypokinesia with LVEF of 35 %	NA	3	Heparin
Case series[19]	LVEF of 50% and hypokinesia of the anterolateral and inferolateral walls and LVEF of 60%, with hypokinetic inferior and inferolateral walls	NA	2–7	PCI
Case[20]	Inferior wall motion abnormality and preserved LVEF	NA	3	PCI

MRI: magnetic resonance imaging; LVEF: left ventricle ejection fraction; LGE: late gadolinium enhancement; NA: not applicable; NSAIDs: nonsteroidal anti-inflammatory drugs; PCI: percutaneous coronary intervention.

In-hospital treatment and outcomes

No case of acute fulminant myocarditis was reported after COVID-19 vaccination. Most reported AM cases were hospitalized for 3–5 days on average, and all of them were discharged uneventfully[2-5,7-9,11,14,15]. In the treatment of AM, high-dose aspirin, colchicine, beta-blockers, and steroids were most preferred[1-5,12,14]. In addition to anti-ischemic and anti-aggregant therapy, the primary percutaneous coronary intervention was performed in patients presenting with AMI[16-20]. All AMI cases were discharged uneventfully, except the 86-year-old male patient who did not survive during the in-hospital course[16].

DISCUSSION

AM is generally regarded as an uncommon adverse effect following vaccination. According to reports, the majority of previously documented post-vaccine AM cases were subclinical and were discovered by routine pre- and post-vaccine troponin level assessments[21]. However, in our review, all the cases documented following COVID-19 immunization were symptomatic. This implies that asymptomatic individuals might not be identified, and as a result, cardiac events following immunization might be significantly greater than predicted.

Although the causes of AM due to COVID-19 vaccinations are not well understood, several potential pathophysiological explanations have been proposed. It has been considered that in some people with genetic vulnerability, the immunological response to mRNA-based COVID-19 vaccines may be uncontrollable, resulting in the activation of an abnormal innate and acquired immune response[22]. Also, both dendritic cells and Toll-like receptor-expressing cells subjected to mRNA may still be able to produce cytokines in certain people, albeit this may be significantly decreased when exposed to mRNA with nucleoside alterations as opposed to unmodified RNA[22]. As a result, the immune system may recognize the mRNA as an antigen, leading to hyperactivation of the inflammatory and immunologic pathways, which may have a role in the occurrence of AM in certain people as part of a systemic response[22].

During vaccination, an allergic reaction may develop, which can be classified as a vaccine-related adverse effect. It is always difficult to determine whether a response is caused by the vaccination or by other causes. Adjuvants are usually included in the vaccines to enhance stability, solubility, and absorption, which can result in IgE-mediated anaphylactic responses following immunization. This might be one explanation for AMI following the COVID-19 vaccination. The fact that all published AMI cases had their complaints started within a short time after the initial dosage of vaccination supports this hypothesis. Another potential AMI cause, as proposed by Warkentin et al., is vaccine-induced prothrombotic immune thrombocytopenia, which is similar to heparin-induced thrombocytopenia and leads to thrombotic manifestation[23].

Future perspective

The number of documented cases supports the "very uncommon" interpretation of vaccine-related cardiac side effects despite the fact that hundreds of millions of COVID-19 vaccinations have been administered globally. It was also clearly demonstrated that the majority of the patients with cardiac adverse events demonstrated full recovery in terms of both symptoms and imaging. Moreover, it must be highlighted that since there has been no causative link between COVID-19 vaccinations and cardiac events, the effectiveness of the COVID-19 vaccination far exceeds some possible drawbacks. Consequently, more research on AM, AMI, and other cardiac events before and after COVID-19 vaccination will enrich the literature about the long-term effects of the vaccination and determining the incidence rate.

AUTHORS' CONTRIBUTIONS

TÇ: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. **MIH:** Conceptualization, Formal Analysis, Writing – review & editing. **ALO:** Supervision, Formal Analysis, Writing – review & editing. **VÇ:** Data curation, Funding acquisition, Resources, Writing – review & editing. **MS:** Data curation, Funding acquisition, Resources, Writing – review & editing. **SY:** Funding acquisition, Resources, Writing – review & editing.

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ORIGINAL ARTICLE
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Clinical implication of preoperative psoas muscle area in endometrial cancer patients

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SUMMARY

OBJECTIVE: Obesity is a significant risk factor for endometrial cancer. In contrast, sarcopenia describes a loss of the body's muscle mass that is closely related to unfavorable clinical outcomes. Even endometrial cancer patients have high rates of obesity, and they should have a significantly higher risk for undiagnosed sarcopenia or fragile muscle quality.

METHODS: This is a retrospective study that included an endometrial cancer database collected from a tertiary gynecologic cancer center. We investigated the relationship between preoperative psoas muscle area by magnetic resonance imaging, surgical outcomes and pathological features.

RESULTS: The study included 116 patients, the mean height was 160 cm (Standart deviation 7), weight was 72 kg (Standart deviation 18), and the median duration of hospitalization was 4 days (Interquartile range 2–9) in the whole study group. Sarcopenia was diagnosed in 25 (21.6%) patients, according to the magnetic resonance imaging findings. Three (6.5%) obese patients had sarcopenia, but it was 31.4% in nonobese patients (p=0.026). The median duration of hospitalization was five days (3–9 days) in the sarcopenia group, and it was four days (2–7 days) in the non-sarcopenia group.

CONCLUSION: Sarcopenic patients did not have increased surgical complication rates following uterine cancer surgery. We should be aware of hospitalization duration in those patients, and sarcopenic counterparts necessitate longer follow-up after the surgery.

KEYWORDS: Endometrial cancer. Sarcopenia. Stay lengths. Histopathology.

INTRODUCTION

The most common gynecologic cancer is endometrial cancer in women, and more than 10,000 deaths are expected in the USA per year[1]. Many reproductive risk factors such as early age of menarche and elder age of menopause, nulliparity, infertility, the first age of delivery elder than 30 years old, and drugs such as tamoxifen for breast cancer prevention have been associated with a higher risk of developing endometrial cancer[2]. Also, obesity is a significant risk factor for endometrial cancer, and it has an increased relative risk in the disease incidence at least threefold[1]. In addition to the cancer risk, obesity causes poorer oncological outcomes than normal-weight patients[3].

Sarcopenia describes a loss of the body's muscle mass that can cause chronic disease, cancer, and advanced age and is closely related to unfavorable clinical outcomes[4]. In sarcopenia, skeletal muscle changes can exist independently from body mass index (BMI) and usually cannot be clinically diagnosed, especially in obese cancer patients[5]. Decreased muscle mass has been linked to poorer oncological outcomes related to radiodensity and is called myosteatosis. Also, it is associated with worse surgical complications[6]. High rates of obesity in endometrial cancer patients can have a significantly higher risk for undiagnosed sarcopenia or fragile muscle quality. It may be associated with poorer outcomes. That is why we want to investigate sarcopenia and its possible clinical implications in endometrial cancer patients with FIGO stages I–III.

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METHODS

Patient selection

Following the local institutional review board's approval, all medical records of patients with endometrial cancer, proven histologically, were reviewed. Patients who had evidence of distant disease in preoperative imaging were excluded from the study. We recorded demographic features such as age, stage, baseline laboratory parameters, BMI, weight, and magnetic resonance imaging (MRI) findings for sarcopenia from the hospital records. The surgical procedure included total hysterectomy, omentectomy, peritoneal washing, and lymph node dissection if needed. After the surgery, all patients were referred to medical and radiation oncology clinics for adjuvant treatment planning. BMI was calculated with the following formula: the patient's weight (kg) divided by height (m) squared. According to the National Institutes of Health (NIH) criteria, all patients were grouped as obese (BMI>30) and nonobese (BMI<30)[7].

Psoas muscle area measurement

Patients who underwent preoperative upper abdominal MRI were included in the study. The study included 116 patients, and an abdominal MRI was performed in a preoperative setting. MRI was performed with a 1.5 T (Siemens, Magnetom Aera, Erlangen, Germany) unit with a 4-channeled body coil. Preoperative axial MR images of the patients were transferred to the open-access software Image J program and evaluated by a 12-year-experienced abdominal radiologist. The psoas muscle area (PMA) was calculated in the sections passing through the L3 vertebral body where both transverse processes were seen. The right and left psoas muscle borders were drawn manually by the same radiologist (Figure 1). The areas of the right and left psoas muscles were calculated separately with the Image J program and summed. Based on data published by Golse et al.[8] in 2017 and Farkas et al.[9] in 2019, sarcopenia was defined as a PMA<1561 mm^2 for males and <1464 mm^2 for females.

Statistical analysis

Demographics and pathological features were stratified as the groups with sarcopenia or normal weight by χ^2/Fisher's exact test for categorical data and Student's *t*-test for continuous data (Table 1).

A normality test was performed for all data and determined with standard deviation or interquartile range as appropriate. All analyses were done with SPSS version 22.0 (IBM Corporation, New York, USA). p<0.05 were considered statistically significant.

RESULTS

The whole study group's mean age was 61.7 years old (sd 10), and the BMI was 28 (SD 7). The most common histological subtype was endometrioid adenocarcinoma in 103 (89%) patients. The mean height was 160 cm (SD 7), the mean weight was 72 kg (SD 18), and the median duration of hospitalization was 4 days (IR 2–9) in the whole study group.

Total PMA was calculated as the sum of left and right PMA values. The median left PMA was 1420 mm^2 (461–4659), the median right PMA was 1393 mm^2 (452–4865), and the total PMA was 2867 mm^2. Sarcopenia was diagnosed in 25 (21.6%) patients, according to the MRI findings. Three (6.5%) obese patients had sarcopenia, but it was 31.4% in nonobese patients (chi-square p-value 0.026). When we analyzed diabetic patients, they had a 31.7% sarcopenia prevalence, but nondiabetic patients had 16% (p-value 0.002). The regression analysis included FIGO stage, myometrial, serosal, lymphovascular, perineural invasion, lymph node metastasis, and tumor grade, but it did not show a significant correlation with PMA measurements (p-values were 0.49, 0.6, 0.8, 0.7, 0.32, 0.16, and 0.3, respectively) (Table 2).

Postoperative complications such as 90-day mortality, surgical site infection, ileus, urinary tract infection, and hospitalization duration were recorded in the study, and PMA values significantly correlated with hospitalization duration (p=0.001). The median duration of hospitalization was five days (3–9 days) in the sarcopenia group, and it was four days (2–7 days) in the non-sarcopenia group.

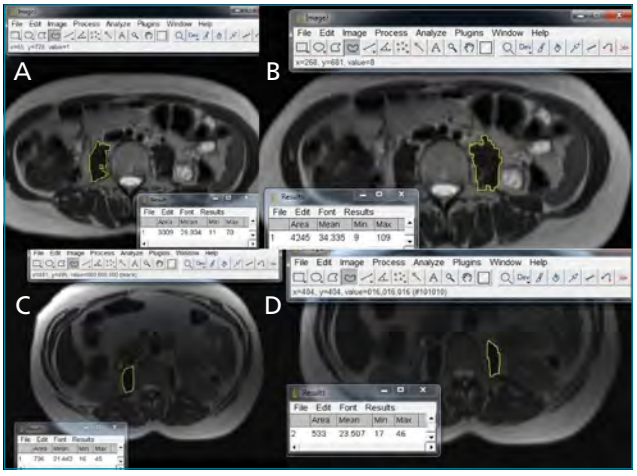

Figure 1. Manual psoas muscle area measurement is seen using preoperative magnetic resonance imaging scans. Measurement of right and left psoas muscle area are seen **(A, B)** in a 43-year-old female patient's axial T2 magnetic resonance imaging scan. Her total psoas muscle area is within normal limits. **(C, D)** Sarcopenia is detected in a 58-year-old female patient's axial T2 magnetic resonance imaging scan. Her psoas muscle area is 1269 mm^2 (less than 1464 mm^2).

DISCUSSION

This study investigated preoperative sarcopenia incidence with MRI and its possible correlation with the pathological features of endometrial cancer. Our results showed that many pathological parameters did not differ between sarcopenia and non-sarcopenia patients. Only one parameter, i.e., the duration of hospitalization, was significantly higher in the sarcopenia group.

Obesity is one of the common risk factors for endometrial cancer and causes nearly threefold relative risk. Otherwise, obesity affects the prognosis of endometrial cancer and poorer outcomes than normal-weight counterparts[10]. Sarcopenia is a different condition with muscle mass loss and/or decreased muscle quality. It negatively impacts cancer patients, including postoperative complications and lower chemotherapy tolerance[11]. Sarcopenia in pancreatic and lung

Table 1. Pathological characteristics of the patients.

Pathological characteristics	Number of patients (%)
FIGO stage	
Stage I	44 (38)
Stage II	52 (49)
Stage III	20 (17)
Myometrial invasion	
Lower than ½	61 (53)
Higher than ½	55 (47)
Cervical stromal invasion	
Negative	102 (88)
Positive	14 (12)
Lymphovascular invasion	
Negative	95 (82)
Positive	21 (18)
Serosal invasion	
Negative	108 (93)
Positive	8 (7)
Lymph node metastasis	
Negative	102 (88)
Positive	14 (12)
Perineural invasion	
Negative	114 (98)
Positive	2 (2)
Tumor grade	
Grade I	46 (40)
Grade II	43 (37)
Grade III	27 (23)

Table 2. Characteristics and anthropometrics of patients with endometrial cancer stratified by sarcopenia and non-sarcopenia.

Pathological characteristics	Sarcopenia, n	Non-sarcopenia, n	p-value
FIGO stage			
Stage I	8	36	0.77
Stage II	12	40	
Stage III	5	15	
Myometrial invasion			
Lower than ½	12	49	0.6
Higher than ½	13	42	
Cervical stromal invasion			
Negative	24	78	0.16
Positive	1	13	
Lymphovascular invasion			
Negative	21	74	0.75
Positive	4	17	
Serosal invasion			
Negative	23	85	0.8
Positive	2	6	
Lymph node metastasis			
Negative	24	78	0.16
Positive	1	13	
Perineural invasion			
Negative	24	90	0.32
Positive	1	1	
Tumor grade			
Grade I	7	39	0.40
Grade II	11	32	
Grade III	7	20	

cancer patients was broadly investigated in the literature. A recent study showed poorer overall survival in patients with pancreatic cancer who had sarcopenia. The study measured CT-based imaging and included a preoperative setting. Thereby, 90% of patients had early-stage pancreatic cancer[5]. Similar to our results, they did not find a significant correlation between sarcopenia and pathological parameters including tumor grade, lymphovascular invasion, and perineural invasion. Portal et al. found that sarcopenia has a significantly lower survival in patients diagnosed with lung cancer[12]. With respect to surgical complications, a meta-analysis revealed that preoperative sarcopenia is associated with prolonged hospital stay following pancreatic cancer surgery. Still, it did not have a negative impact on postoperative morbidity[13].

There is scarce literature about sarcopenia in gynecologic cancers; even if most gynecologic cancer patients can be overweight, sarcopenia is not uncommon in this group. A review about sarcopenia incidence showed a wide range from 11 to 74% overall population and from 1 to 36% in the setting of obesity[14]. Moreover, sarcopenic visceral obesity is a different condition than sarcopenia, and it is characterized as increased visceral fat with decreased muscle mass. Further, it can cause higher postoperative mortality, surgical complications, and lower survival rates[15,16]. In our study, obese patients had a 6.5% of sarcopenia rate, which was concordant with the literature; on the other hand, they showed a similar postoperative complication rate compared with sarcopenic patients with normal weight. A few studies investigated sarcopenia in endometrial cancer, measuring sarcopenia with CT-based imaging, although they showed poorer oncological outcomes in sarcopenic patients, none of them investigated postoperative complications. Our study was the first MRI-based imaging research for sarcopenia and uterine cancer. The main result of our study was increased hospital stay in patients with sarcopenia.

Also, we have some major limitations such as deficiency of oncological outcomes as overall survival, disease survival, or recurrence rates; thereby, short follow-up period, we could not analyze these parameters. Actually, we did not aim because our focusing intent was postoperative complications.

CONCLUSION

Sarcopenic patients did not have increased surgical complication rates following uterine cancer surgery. We should be aware of hospitalization duration in those patients, and sarcopenic counterparts necessitate longer follow-up after the surgery.

AUTHORS' CONTRIBUTIONS

FB: Conceptualization, Writing – original draft. **DTA:** Data curation. **EO:** Formal Analysis. **BE:** Formal Analysis, Writing – review & editing.

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Changes in cardiac cells due to ticagrelor and enoxaparin in a rat ischemia/reperfusion model

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SUMMARY

OBJECTIVE: Studies on ischemia/reperfusion injury remain the focus of interest. Ticagrelor and enoxaparin, which are antiaggregant and anticoagulant drugs developed for use in many cardiovascular pathologies, are still included in many ischemia/reperfusion studies. Remarkably, their new protective effects, especially with regard to ticagrelor, continue to be reported in the current literature. The aim of this study was to evaluate the beneficial effects of ticagrelor and enoxaparin pretreatments on the rat heart with histological and immunohistochemical markers in an ischemia/reperfusion model.

METHODS: Wistar–albino rats (weighing 350–400 g) were divided into four groups as follows: Sham-Control (Group 1), Control-Saline+ischemia/reperfusion (Group 2), Ticagrelor+ischemia/reperfusion (Group 3), and Enoxaparin+ischemia/reperfusion (Group 4). The ischemia/reperfusion injury model was applied to Group 2, Group 3 and Group 4. Heart tissue sections were stained with hematoxylin and eosin for histological examinations. Caspase 3 immunostaining was evaluated to detect apoptosis in the heart tissue sections.

RESULTS: Both pretreatments ameliorated the ischemic damage but especially tissue sections belonging to Group 3 were nearly similar to control levels. The results indicated that ischemia/reperfusion-induced myocardial damage was significantly increased in Group 2, whereas ticagrelor and enoxaparin pretreatments in Group 3 and Group 4 significantly decreased apoptotic scores and the histological appearance of the Group 3 close to the normal myocardium ($p<0.001$).

CONCLUSION: As supported by histological findings in our study, ticagrelor and enoxaparin have protective properties for heart tissue in this ischemia/reperfusion injury model.

KEYWORDS: Ticagrelor. Enoxaparin. Ischemia. Rats. Reperfusion injury.

INTRODUCTION

"Ischemia" is defined as the loss of blood flow by depriving a tissue of metabolic substrates such as glucose as a result of reduced blood flow and impaired nutrition. "Reperfusion" is used to describe the restoration of blood flow and tissue nutrition after a period of ischemia and is a critical step in dealing with any ischemia condition[1,2]. Ischemia is associated with progressive decline in cellular functions, increased oxidative and inflammatory response. Increased permeability, disruption of extracellular matrix, increased proteases, impaired intravascular laminar flow, and the relationship among damaged endothelial-thrombus aggregation, leukocyte adhesion, foam cells, and ischemia/reperfusion (I/R) injury have been clarified at present[1]. In addition to their current primary effects in

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cardiovascular therapy and protection, many pharmacological agents provide clues as to where the protective effects will be located in experimental animal studies. Therefore, studies on I/R injury remain the focus of interest.

Ticagrelor and enoxaparin, which are antiaggregant and anticoagulant drugs developed for use in the treatment of many cardiovascular pathologies, occlusive and ischemic cardiovascular diseases, myocardial infarction (MI), some arrhythmias, ischemic stroke, valve surgery, and coronary bypass surgery, are still included in many I/R studies. Remarkably, the new protective effects of ticagrelor (it significantly prevents depolarization of mitochondrial membrane potential and increases in reactive oxygen species (ROS) with a marked increase in the ATP level in insulin-resistant H9c2 cells and reverses the increases in the resting level of free Ca2+ and mRNA level of P2Y12 receptors as well as preserved endoplasmic reticulum stress and apoptosis) and enoxaparin (it reduces cell proliferation and tumor cell migration by inhibition of protease-activated receptor-1 [PAR-1]) continue to be reported in the current literature[3-5].

With the increase in the number and quality of qualified experimental basic education and research centers in recent years, immunohistochemical laboratory studies shed light on clinical studies. In this way, the protective effects of pharmacological agents on cells and systems became more important. It should be emphasized that the effects of many pharmacological agents on inflammation, apoptosis and I/R injury have been better studied with multidisciplinary precursor animal experiments conducted in these qualified centers.

The aim of this study was to evaluate the beneficial effects of ticagrelor and enoxaparin pretreatments on the rat heart with histological and immunohistochemical markers in an I/R model.

METHODS

Animal preparation and experimental design

All experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). The experiments reported in this study were conducted in accordance with the Regulation of Animal Research Ethics Committee in Turkey (July 6, 2006, Number 26220). The ethical approval was granted by the Kocaeli University Animal Experiments Ethics Committee. All experiments were conducted between 9:00 a.m. and 12:00 p.m. under standard laboratory conditions (22±2°C room temperature; 12 h light/dark cycle with lights on at 7:00 a.m.). Tap water and food pellets were provided ad libitum for animals.

Thirty-six mature male Wistar–albino rats (weighing 350–400 g) were divided into four groups as follows: Sham-Control (Group1), Control-Saline+I/R (Group 2), Ticagrelor+I/R (Group 3), and Enoxaparin+I/R (Group 4). The I/R injury model was applied to Group 2, Group 3, and Group 4 induced by clamping the aorta infrarenally for 2 h followed by 4 h of reperfusion after anesthetized with intraperitoneal ketamine hydrochloride (Ketalar, Pfizer, Istanbul, Turkey). Cessation of arterial flow was confirmed by means of the absence of an audible continuous-wave Doppler signal. Group 1 was not treated. Before the ischemic period, Group 2 received 0.1 mL/kg saline, Group 3 received 25 mg/kg ticagrelor (Brilinta, AstraZeneca, Södertalje, Sweden) *via* gastric gavage while Group 4 received 0.75 mg/kg enoxaparin (Clexane, Sanofi Winthrop Industrie, Maisons-Alfort, France) subcutaneously[6,7].

At the end of the experiment, rats were sacrificed by the lethal dose of sodium thiopental (Pentothal Sodium, Abbot, Italy). The hearts of all rats were excised through midline sternotomy, washed with 0.9% saline solution, and then fixed in 10% neutral buffered formalin for histological tissue processing.

Hematoxylin and eosin staining

Heart tissue samples were dehydrated in graded series of ethanol, cleared in xylene, and embedded in paraffin blocks. Notably, 4 μm-thick sections were stained with H&E for histological examinations. Degeneration of cardiomyocyte arrangement, heterogeneity of sarcoplasm, interfibrillar distance, necrosis, and increase in capillaries were assessed.

Caspase-3 immunohistochemistry

Caspase-3 immunostaining was evaluated to detect apoptosis in the heart tissue sections. In brief, tissue sections were deparaffinized with xylene and then rehydrated. Following the antigen retrieval step, the sections were incubated in 3% H_2O_2 for 15 min to block endogenous peroxidase activity.

After blocking serum, the anti-caspase-3 monoclonal antibody (74T2, Life Technologies/Thermo, 1:100 dilution) was added to the sections and incubated overnight at room temperature. After washing with phosphate-buffered saline (PBS), the biotinylated secondary antibody (ab80437, Abcam) was added to the sections and incubated for 20 min. Then, the sections were washed with PBS and incubated with horseradish peroxidase (HRP)-labeled streptavidin at room temperature for 15 min. The samples were developed using 3,3′-diaminobenzidine (DAB) chromogen according to the manufacturer's standard procedure. Negative control staining was applied by omitting the primary antibody. The sections were counterstained using hematoxylin for 1 min and examined at 200× and 400× magnification using a light microscope. Positive cells displayed

a brown–yellow coloration. The approximate percentage of apoptotic cells was estimated based on 10 representative fields in each section. The immunostaining intensity was scored as negative (–), very weak (1+), moderate (2), strong (3+), and very strong (4+)[7-10].

The total score was based on the percentage of positive cells and the degree of immunostaining intensity. The percentage of positive cells was graded as follows: no stained cells (Grade 0), 1–25% stained cells (Grade 1), 26–50% stained cells (Grade 2), 51–75% stained cells (Grade 3), and 76–100% stained cells (Grade 4) in the representative field. Three observers who were blinded to the groups performed evaluations semi-quantitatively. The total index was calculated using the following formula: total index (percentage of positive cells)×(immunostaining intensity). All slides were examined under a light microscope (Leica DM 1000, Germany), and photomicrographs were taken using an attached camera (Leica DMC 2900, Germany).

Statistical analysis

All statistical analyses were performed using IBM SPSS for Windows version 20.0 (IBM Corp., Armonk, NY, USA). Caspase-3 scoring was analyzed using this software and presented as mean±standard deviation (SD). The Kolmogorov–Smirnov test was used for evaluating the normal distribution of the variables. The difference between the groups was analyzed by using a one-way analysis of variance (ANOVA) for numerical variables with normal distribution. Tests were performed within a 95% confidence interval and $p<0.05$ was considered significant.

RESULTS

Histological results

H&E staining of the myocardial sections of the Group 1 showed normal morphology (Figure 1A), whereas Group 2 exhibited disorganized cardiac muscle fibers, heterogeneity of sarcoplasm, morphologically deformation of nuclei, increase in interfibrillar space, edematous areas in the connective tissue, necrosis, and increased capillary formation (Figure 1B). However, both pretreatments improved and ameliorated the ischemic damage but especially tissue sections belonging to Group 3 were nearly similar to control levels (Figures 1C and 1D).

Caspase-3 immunoreactivity

In addition to morphological findings, caspase-3 immunoreactivity in myocardium sections belonging to the Group 1, Group 2, and pretreatment groups was examined (Figure 2).

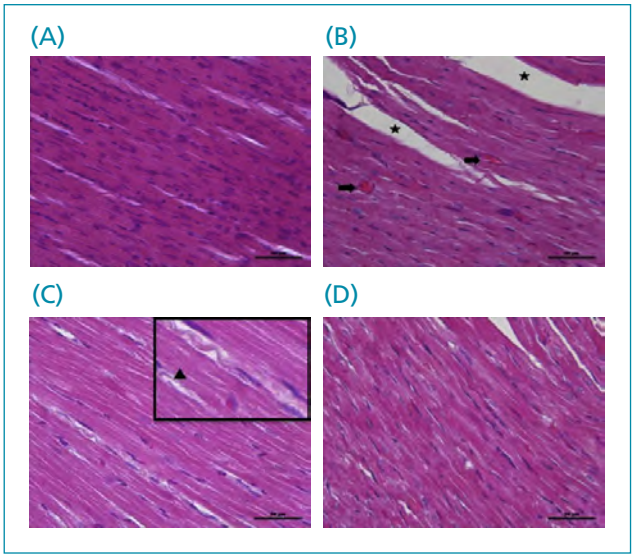

Figure 1. Hematoxylin and eosin staining of the myocardial sections of the Sham-Control **(A)**, Control-Saline+ischemia/reperfusion **(B)**, Ticagrelor+ischemia/reperfusion **(C)**, and Enoxaparin+ischemia/reperfusion **(D)**. Note degeneration of cardiomyocyte arrangement and heterogeneity of sarcoplasm, interfibrillar distance (star), increased capillaries (arrow) in Control-Saline+ischemia/reperfusion group. Arrowhead indicates normal striated appearance. Hematoxylin and eosin 400×.

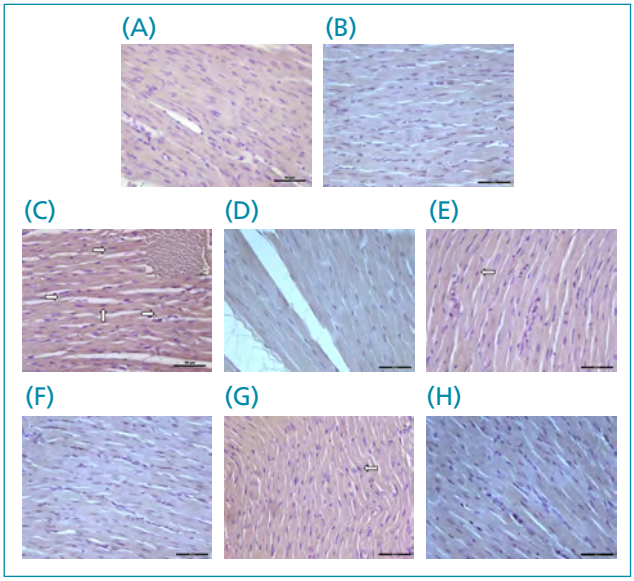

Figure 2. Caspase 3 immunoreactivity of the Sham-Control **(A)**, Control-Saline+ischemia/reperfusion **(C)**, Ticagrelor+ischemia/reperfusion **(E)**, and Enoxaparin+ischemia/reperfusion **(G)** groups with their negative controls **(B, D, F, H)**. In **(C)**, note the longitudinal sections of dark-stained cardiomyocytes and their transverse sections in the upper right box. Arrows indicate the Caspase 3 (+) cardiomyocytes. In right side, no immunoreactivity is observed in negative controls. 400×.

Increased caspase-3 immunoreactivity was noticed in the myocardium sections of Group 2, and caspase-3 immunostaining was weaker in the pretreatment groups (Figure 2).

Caspase-3 expression was significantly higher in Group 2 compared with Group 1 ($p<0.001$), and this expression index was significantly decreased in the pretreatment groups compared with Group 2 ($p<0.001$; Figure 3). For all groups, caspase-3 negative control slides were examined, and no immunoreactivity was observed ($p<0.001$; Figure 3).

Caspase-3 total IHC index was significantly decreased in ticagrelor and enoxaparin pretreatment groups compared with the Control-Saline+I/R group ($p<0.001$). These results indicated that I/R-induced myocardial damage was significantly increased in Group 2, whereas ticagrelor and enoxaparin pretreatments in Group 3 and Group 4 significantly decreased caspase-3 scores (Figure 3).

In conclusion, the apoptotic index with the histological appearance of the Group 3 and Group 4 close to the normal myocardium display that ticagrelor and enoxaparin have effective protective properties for the heart muscle tissue in this I/R injury model.

DISCUSSION

This study showed that ticagrelor and enoxaparin pretreatments have cardioprotective effects on myocardium in the I/R rat model. According to our findings and previous data, ticagrelor possibly increased the extracellular adenosine which is a major mediator of myocardial protection against I/R injury and is essential for myocardial protection by ischemic preconditioning and various pharmacological preconditioning[11].

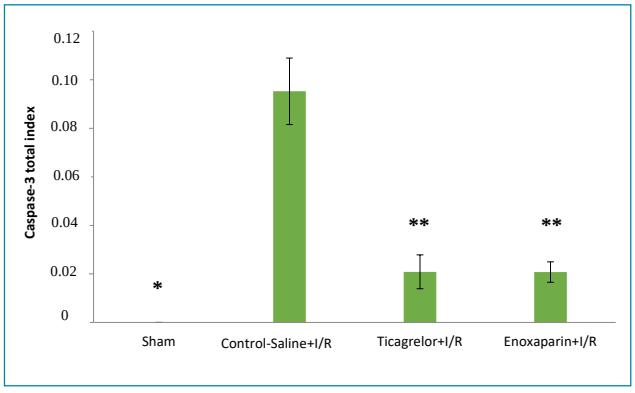

Figure 3. Graphical representation of Caspase-3 total index. While caspase-3 total index was significantly higher in Group 2 compared with Group 1 (*$p<0.001$), this index was significantly decreased in the pretreatment groups (Group 3 and Group 4) compared with Group 2 (**$p<0.001$).

Abdominal aortic cross-clamping is often used for I/R injury in experimental animal models. I/R injury affects not only the kidney, testes, and lower extremity distal to the clamped abdominal aorta but also proximal and distant tissues such as the brain, heart, and lungs, with a series of inflammatory cascades triggered[12]. The mechanisms that cause I/R injury after ischemia are as follows:

(i) increased amount of Ca^{2+} ions entering the myocardial cells in the ischemic process;
(ii) the excessive amount of hypoxanthine accumulated in the issue as a result of adenosine triphosphate (ATP) breakdown during the ischemic period, to form excess superoxide by xanthine oxidase and the formation of free oxygen radicals; and
(iii) increased thromboxane-A2 level.

Reperfusion is responsible for major lesions seen in the cells of the ischemic organ. Systemic complications, such as adult respiratory distress syndrome and kidney and liver dysfunction, can be seen with this cellular damage. Occasionally, these negative effects are seen through the systemic blood circulation, by leukocytes, proinflammatory cytokines, adhesions, and reactive oxygen metabolites, affecting more than one distant organ and systems[13-16]. We know that the endothelium helps to prevent cell–cell interactions between blood-borne inflammatory cells (i.e., leukocytes and platelets). Adenosine and nitroxide (NO^-), secreted from the endothelium and showing antineutrophil and antiplatelet effects, cannot be secreted sufficiently from the endothelium as a result of I/R damage. Inflammatory reactions with free oxygen radicals and proteases are further increased with resisting neutrophilic activation and proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, and complement activity such as C3a, C5a begin the process leading to cell death[17].

The cardiac dysfunction occurs in myocardial ischemia, and the loss of energy substrates in myocardial ischemia leads to the generation of ROS; the high levels of ROS are deleterious and can induce a variety of cardiomyocyte abnormalities[18]. Oxidative stress and the generation of ROS during I/R increase the production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-6, and IL-1β. These cytokines induce apoptosis and cellular damage. ROS are potent oxidizing and reducing agents, which activate neutrophil and lipid peroxidation, leading to cell membrane damage[19]. In the study by Shiroma ME et al.[20], it has been shown that melatonin can support better quality ovarian grafts in inflammatory processes and oxidative stress and explained that the purpose of cell protection is to prevent mitochondrial apoptosis by reducing bcl2 expression in I/R injuries that damage cells and mitochondria[20].

Ticagrelor, a cyclopentyl triazolo pyrimidine, is a direct-acting, selective, and reversibly binding $P2Y_{12}$ receptor antagonist. The $P2Y_{12}$ receptor has a major role in primary hemostasis and in arterial thrombosis. When bound to the $P2Y_{12}$ receptor, ticagrelor prevents adenosine diphosphate (ADP)-mediated platelet activation and aggregation. In addition to the primary effects mediated by $P2Y_{12}$ receptor antagonism, ticagrelor increases local endogenous adenosine levels by inhibiting the uptake of adenosine[21], thereby protecting the extracellular adenosine from its intracellular metabolism. Unlike thienopyridines, ticagrelor is not a prodrug. The antiplatelet effect is independent of the CYP2C19 pathway. In many I/R studies, adenosine and NO^- were used against this cascade in preventing the increased inflammatory response seen during cardiopulmonary bypass, and their positive effects were demonstrated[22]. Thus, by increasing its production and preventing its metabolism, ticagrelor should have beneficial effects on extracellular adenosine levels[23]. Besides, several clinical studies have supported the safety, tolerability, and efficacy of ticagrelor in the setting of stable coronary disease and acute MI.

The platelet inhibition and patient outcomes (PLATO) trial showed that ticagrelor was associated with a lower incidence of cardiovascular mortality, MI, or stroke compared with the alternative $P2Y_{12}$ antagonist clopidogrel in patients with acute coronary syndromes when given on a background therapy of acetylsalicylic acid[24,25]. Although the difference was originally ascribed to better and more consistent platelet inhibition than with clopidogrel, it has been speculated that additional benefit of ticagrelor might be provided *via* an adenosine mediated mechanism[26]. In addition, a *post hoc* analysis of the PLATO trial has suggested that ticagrelor reduces sudden death and has also been shown to lower the risk of recurrent ischemic events, including cardiovascular and coronary heart disease death, in diabetic patients with a history of MI[11].

Enoxaparin is a heparin with low molecular weight, and it is an antithrombotic drug that is used in the treatment of thromboembolism, ischemia, and infarction. Enoxaparin inhibits the conversion of prothrombin to thrombin and reduces the conversion of fibrinogen to fibrin, preventing clot formation. It also reduces coagulation factors and inactivates factor-X, and enoxaparin has been shown to exert powerful antioxidant effects by preventing lipid peroxidation. Furthermore, this drug demonstrated anti-inflammatory benefits. In the study by Shaker et al., enoxaparin treatment was shown to effectively reduce doxorubicin-induced cardiotoxicity by suppressing oxidative stress and inflammation and preventing apoptosis[27]. Shaker et al. showed that in the doxorubicin group, there was an increase in malondialdehyde (MDA), a marker of oxidative stress, and a decrease in total antioxidant capacity, increased levels of inflammatory markers such as TNF-a and IL-1β and an apoptotic marker, caspase-3[27]. Additionally, in this study, although enoxaparin treatment did not completely reverse doxorubicin-induced cardiotoxic damage, it decreased cardiac troponin-I compared with the doxorubicin group and improved cardiomyopathy histopathological scores; it reduced MDA levels, increased the total antioxidant capacity in the rat heart to comparable levels compared with the control group; and it significantly decreased TNF-α, IL-1β, and caspase-3 levels compared with only doxorubicin group[27]. However, there are not enough studies in the literature regarding the effect of enoxaparin on myocardial I/R damage. In our study, only the apoptotic activity/caspase-3 score and histological evaluation and focus on apoptosis at the histological level can be considered as limiting factors.

In contrast, the protective effect of ticagrelor, which we showed in our study, overlaps with the study by Liu X et al., and the cardioprotective effects of ticagrelor might partly be mediated by downregulating galectin-3 expression in infarct area in a rat model of myocardial I/R injury[3].

Myocyte regeneration and death of myocytes occur physiologically. Recent studies of these cellular processes increase in pathological conditions have challenged the idea that the heart is a postmitotic organ. The ideas that cardiac homeostasis can be regulated by multipotent cardiac stem cells are guiding new studies[28]. Due to that, comprehensive research on cardiac stem cells will only be possible with a thorough understanding of the inflammatory response and the I/R cascade.

According to the study by Birnbaum et al.[29], ticagrelor, possibly by inducing a local increase in extracellular adenosine levels, may augment reparative mechanisms that attenuate adverse remodeling and fibrosis, and it may increase cell proliferation and stem cell recruitment. Based on these data, it may provide an alternative approach to cardioprotection and stem cell therapy, as evidenced by improved cardiac function[29].

CONCLUSIONS

Ticagrelor and enoxaparin pretreatments have cardioprotective effects on the myocardium in the I/R rat model. According to our findings and previous data, ticagrelor possibly increased the extracellular adenosine which is a major mediator of myocardial protection against ischemia–reperfusion injury and is essential for the myocardial protection by ischemic preconditioning and various pharmacological preconditioning[5]. However,

more comprehensive experimental and clinical studies should be performed to figure out the underlying mechanisms. In conclusion, these results we showed in our study may guide to the uncovered aspects of the I/R cascade, advanced experimental and cardiac stem cell studies to be performed with anticoagulant drugs and antiaggregant drugs. With this laborious study on the I/R model in rat heart, we hope that the protective effect of ticagrelor and enoxaparin may make an effective contribution to larger-scale studies, particularly cardiac and vascular pathologies.

AUTHORS' CONTRIBUTIONS

OF: Conceptualization, Project administration, Data curation, Investigation, Methodology, Writing – original draft. **OB:** Conceptualization, Project administration, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **YY:** Investigation, Supervision, Validation. **MYY:** Methodology, Resources, Writing – review & editing. **SFR:** Visualization, Writing – original draft. **KKS:** Formal Analysis, Software. **ATK:** Conceptualization, Project administration, Methodology, Supervision.

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ORIGINAL ARTICLE
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Inula graveolens induces selective cytotoxicity in glioblastoma and chronic leukemia cells

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SUMMARY

OBJECTIVE: Crude oil extracts, components of extracts, and ethanolic extracts of *Inula graveolens* possess various pharmacological activities on various cancer cells including antioxidative and antiproliferative effects. Aqueous extract of this species has not been investigated on the liquid malignancies and solid tumors with a high incidence of treatment refractoriness and poor survival outcomes such as glioblastoma and leukemia. Hence, the present study aimed to evaluate the cytotoxic efficiency of *I. graveolens* aqueous extracts on human glioblastoma multiforme and chronic myelogenous leukemia cell lines in comparison to non-cancerous primary rat cerebral cortex and human peripheral blood mononuclear cells.

METHODS: The cells were treated with the extracts of *I. graveolens* (125–1000 µg/mL) for 48 h, the cellular viability was identified using 3'-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, and lactate dehydrogenase release was measured to determine the cytotoxic potential. Total oxidant status and apurinic/apyrimidinic endodeoxyribonuclease 1 assays were used to determine the oxidative status of cells and DNA damage, respectively.

RESULTS: *I. graveolens* showed selective cytotoxicity toward human glioblastoma multiforme and chronic myelogenous leukemia cell lines and exhibited a higher antiproliferative effect against cancer cells in comparison to non-cancerous cells. Moreover, it significantly reduced the apurinic/apyrimidinic endodeoxyribonuclease 1 levels on both cancer cell lines as compared with their control cells without changing the levels of an oxidative stress marker.

CONCLUSION: The extracts of *I. graveolens* have anti-cancer potential on human glioblastoma multiforme and chronic myelogenous leukemia cell lines without causing oxidative stress.

KEYWORDS: Glioblastoma. Myeloid leukemia. Inula. Cell viability.

INTRODUCTION

Inula graveolens (*Dittrichia graveolens*) is an annual plant that is sticky, strongly aromatic, and widely distributed in the Mediterranean area[1]. The essential oil and extracts of *I. graveolens* possess significant antioxidant, antiproliferative, and antibacterial activities, which have been demonstrated by pharmacological studies[2-4]. Previous phytochemical investigations of this plant have revealed the presence of oxygenated monoterpenes with high contents of bornyl acetate, borneol, and τ-cadinol[5]. Besides, chlorogenic acid, quinic acid, hyperoside, protocatechuic acid, and quercetin are the major phenolic compounds of *I. graveolens*. The significant antioxidant capacity of *I. graveolens* has been thought to be related to the high

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abundance of phenolic compounds[6]. Recently, the compounds isolated from *I. graveolens* have been recorded as an important antiradical power[7]. Moreover, the methanolic extract of *I. graveolens* has been shown that the antioxidant activity helps ameliorate the oxidative stress/antioxidant status balance in elite athletes during a competition period[8]. No recent reports are available about the antiproliferative potential aqueous extract of this species the liquid malignancies and solid tumors with a high incidence of treatment refractoriness and poor survival outcomes such as glioblastoma and leukemia. Therefore, the present study was conducted to clarify the anticancer potential of aqueous extract of *I. graveolens* on human glioblastoma multiforme (U-87 MG) and chronic myelogenous leukemia (K562) cancer cells concerning non-cancerous primary rat cerebral cortex (PRCC) and peripheral blood mononuclear cells (PBMCs).

METHODS

U-87 MG (ATCC® HTB-14) and K562 (ATCC® CCL-243) cell lines were obtained from American Type Culture Collection. The PRCC culture was obtained from the Department of Medical Pharmacology of Ataturk University, Erzurum, Turkey. The PBMCs were obtained from healthy volunteers. Whole blood was collected in the heparin blood tube and transferred to a conical tube containing 4 mL of phosphate-buffered saline (PBS). The diluted blood sample was carefully added onto a new conical tube containing 8 mL of Lymphoprep solution, and the blood was layered over the Lymphoprep solution. After centrifugation, the upper layer was aspirated carefully without disturbing the mononuclear cell layer containing lymphocytes, monocytes, and platelets at the interphase. The mononuclear cell layer was put into a new tube filled with PBS and centrifuged. The supernatant was discarded for the removal of platelets. The pellet was suspended in the RPMI medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were disseminated into 24-well culture plates and cultured in 5% CO_2 and 95% moisture at 37°C for two days.

The aerial parts of *I. graveolens* were infused in distilled water at 98°C of temperature for 15 min and filtered. After sterilization, the infusion was diluted with a culture medium. Cell lines were grown in DMEM supplemented with 1% penicillin-streptomycin solution, 2 mM L-glutamine, and 10% heat-inactivated FBS. Cells were grown to 80% confluency, and 10,000 cells/well were seeded into 96-well plates in triplicate. After 24 h of incubation, the medium was replaced with a fresh medium containing various concentrations (125, 250, 500, and 1000 µg/mL) of *I. graveolens* according to our preliminary study.

After 48 h of treatments, the medium was removed and replaced with 150 µL of 3'-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) stock solution (Sigma Chemical Company, St. Louis, USA). The plates were placed in an incubator at 37°C for 4 h and then MTT was replaced with 150 µL of dimethyl sulfoxide. The absorbance was measured at 570 nm in a microplate reader. Lactate dehydrogenase (LDH) released from damaged cells was quantified by using a kit (Cayman Chemical Company, USA). The rate of NADH reduction directly proportional to LDH activity was measured as an increase in the absorbance at 490 nm.

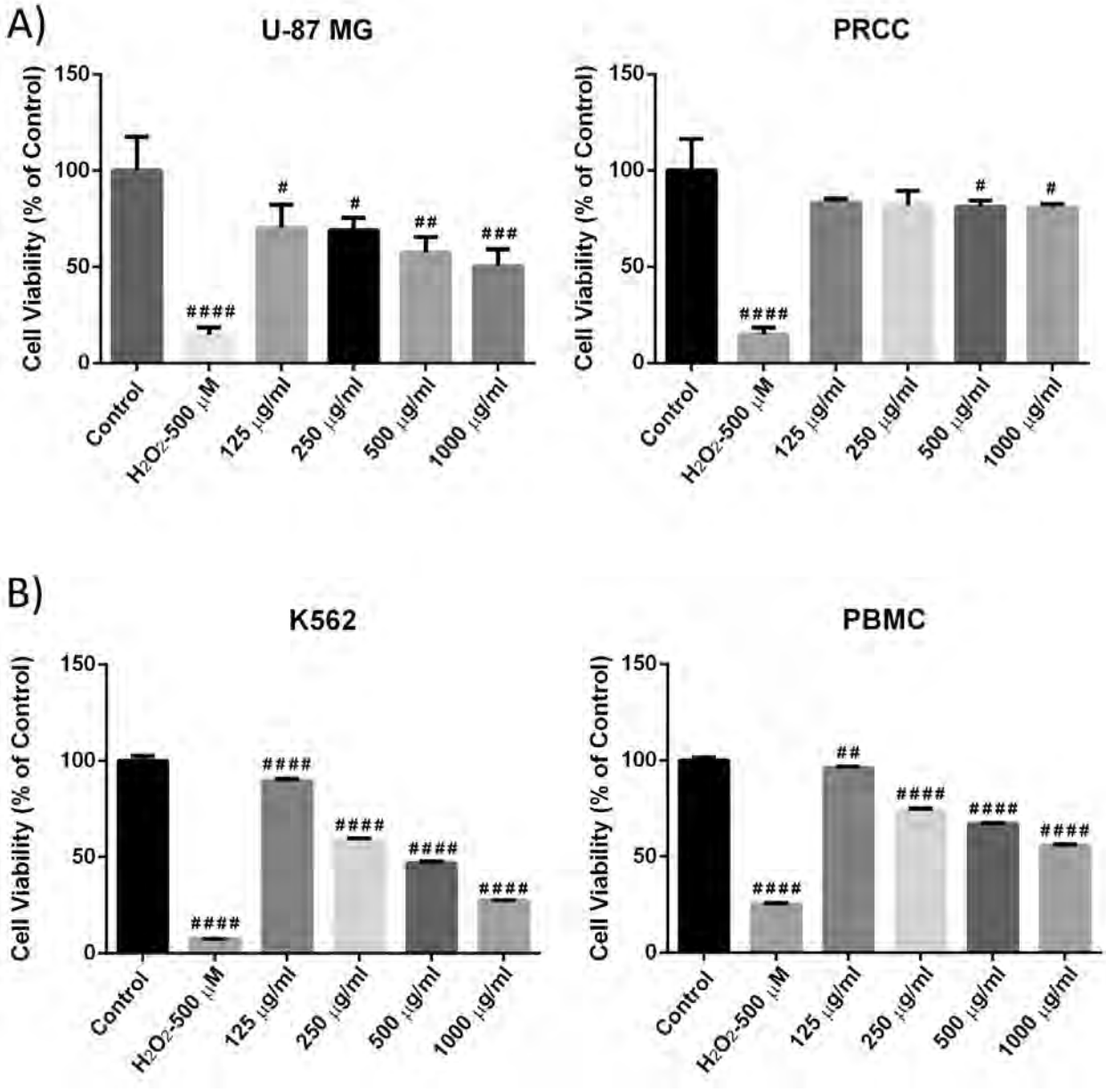
The total oxidant status (TOS) of cultured cells was measured using a commercial Rel Assay Diagnostics® Kit (Turkey). The amount of TOS in culture media was measured by the density of color change at 530 nm.

Apurinic/apyrimidinic endodeoxyribonuclease 1 (APE1) level was examined from cell culture supernatants (ab207616, Abcam, UK). The absorbance (OD) was read at 450 nm, and the concentration of the target in the samples was calculated by constructing a standard curve prepared from standard samples.

To compare the statistical significance of differences between the obtained results of the negative control and treated groups of *I. graveolens* aqueous part, statistical analysis was performed using a one-way analysis of variance (ANOVA) test followed by GraphPad Prism 6.0 statistics software (GraphPad, La Jolla, CA, USA). Tukey's test was used as a *post hoc*. Comparisons among the groups were represented as the mean±standard deviation (SD). A $p<0.05$ was considered statistically significant.

RESULTS

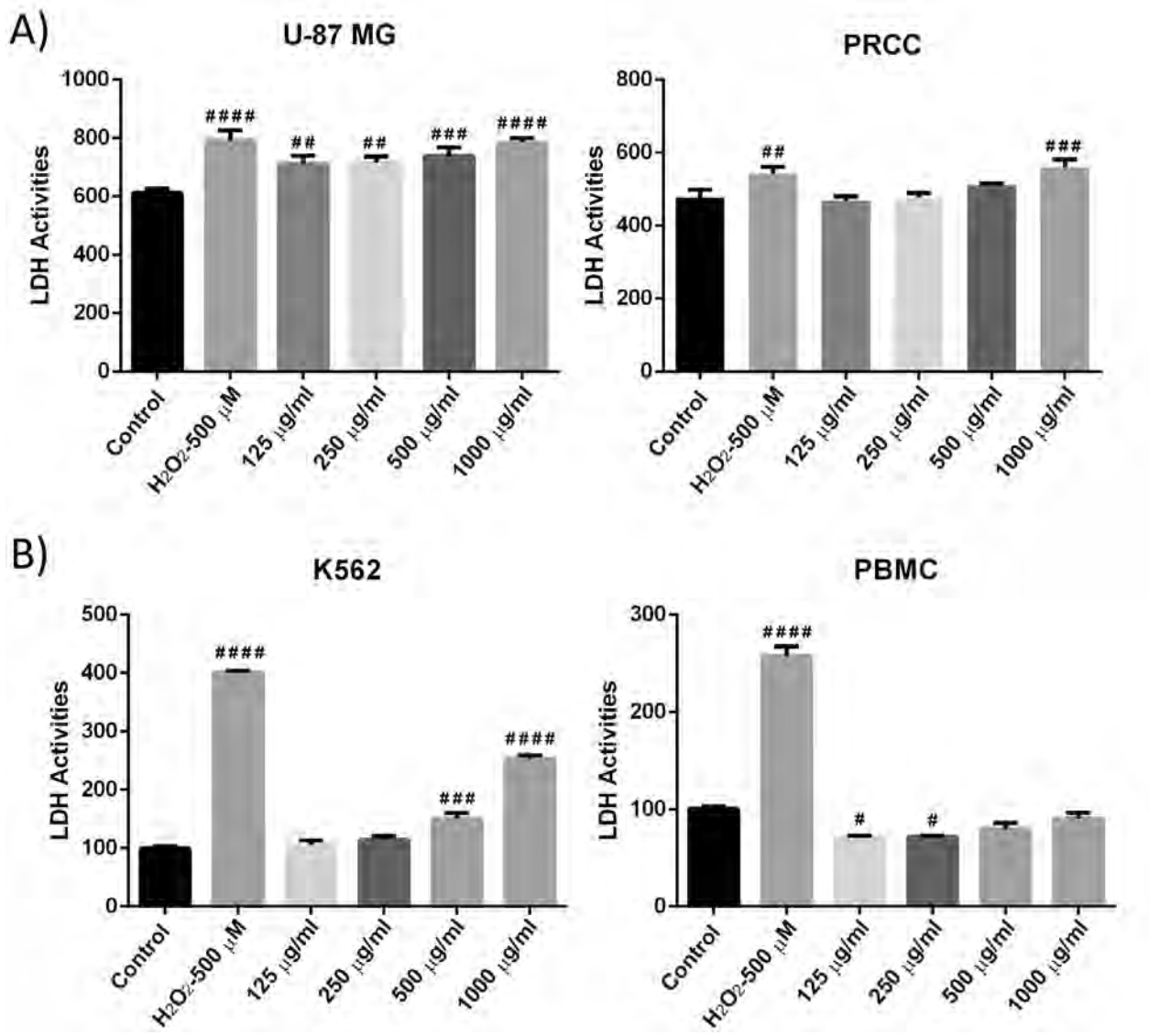
The cytotoxicity of the aqueous extracts of *I. graveolens* against human brain and leukemic cancer cells and non-cancerous cells was detected by MTT and LDH release assay. *I. graveolens* elicited a significant decrease in cell viability in both U-87 MG and K562 cell lines, which occurred in a dose-dependent manner. The extracts at the concentrations of 500 and 1000 µg/mL depicted considerable antiproliferative activity on U-87 MG cell lines ($p<0.01$, reduction of 43% and $p<0.001$, reduction of 50%, respectively). However, more than 80% of PRCC remained viable after treatment with 500 and 1000 µg/mL of the extract ($p<0.05$), indicating that *I. graveolens* exhibited low cytotoxicity against PRCCs. Furthermore, the exposure to *I. graveolens* at the doses of 500 and 1000 µg/mL resulted in the 53% ($p<0.0001$) and 73% ($p<0.0001$) decrease in cell viability on K562 cells, but it is less toxic against healthy blood cells (Figure 1B). The same doses of the extract resulted in 33% ($p<0.0001$) and 44% ($p<0.0001$) decrease in cell viability on PBMCs. Treatment with 1000 µg/mL *I. graveolens*



U-87 MG: Human glioblastoma multiforme; PRCC: primary rat cerebral cortex; K562: Chronic myelogenous leukemia; PBMC: peripheral blood mononuclear cells. #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 indicate statistically significant differences when compared to nontreated cells or cells treated with other concentrations.

Figure 1. Cytotoxic effects of the *I. graveolens* extract against cell lines: (A) Human glioblastoma multiforme and primary rat cerebral cortex and (B) Chronic myelogenous leukemia and peripheral blood mononuclear cells after 48 h of incubation. The cellular viability was determined as the percentage of absorbance of *I. graveolens* treated cultures compared with those of untreated control cells, used as a negative control. Cells treated with 500 µm H_2O_2 were used as a positive control. Values are expressed as mean±standard deviation of three independent replicates.

resulted in more than 50% viable PBMCs and PRCCs. Moreover, *I. graveolens* led to a remarkable increase in the LDH level of U-87 MG cells, while concentrations below 1000 µg/mL did not cause any change in PRCCs ($p<0.001$). The extract at 500 and 1000 µg/mL LDH release significantly increased in K562 cells ($p<0.001$ and $p<0.0001$, respectively), while at the same concentrations, it did not cause any change in the LDH release of PBMCs (Figure 2). Thus, brain cancer and leukemic cells were more sensitive to *I. graveolens* than normal cells, implying that *I. graveolens* could damage the membrane



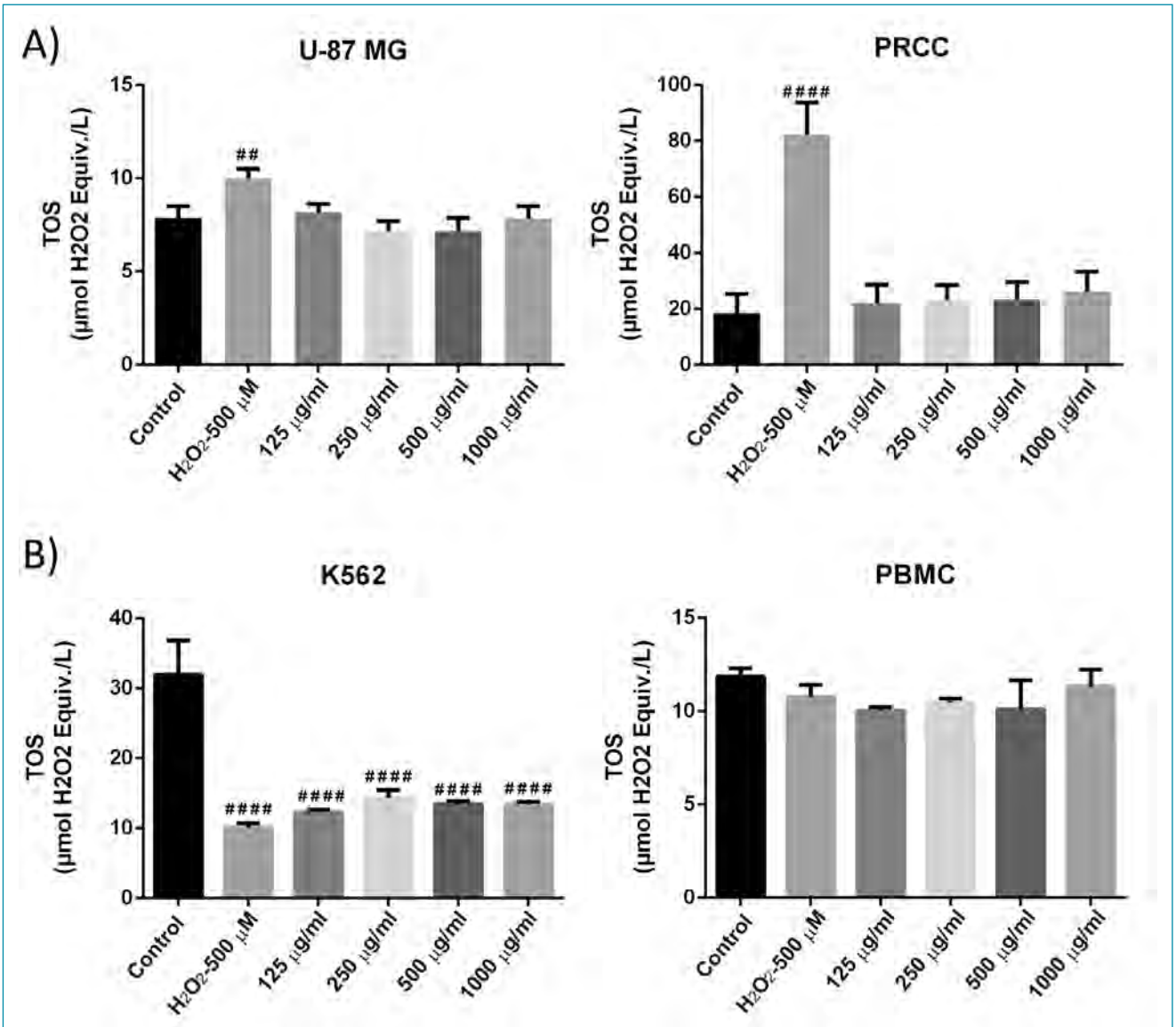
U-87 MG: human glioblastoma multiforme; PRCC: primary rat cerebral cortex; K562: chronic myelogenous leukemia; PBMC: peripheral blood mononuclear cells.

Figure 2. Effects of the *I. graveolens* extract on lactate dehydrogenase release in cell lines (A) Human glioblastoma multiforme and primary rat cerebral cortex and (B) Chronic myelogenous leukemia and peripheral blood mononuclear. Values are expressed as mean±standard deviation of three independent replicates. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ indicate statistically significant differences when compared to non-treated cells or cells treated with other concentrations.

integrity and resulting in dose-dependent induction of cellular toxicity.

Whether the extract from *I. graveolens* induced oxidative stress on the cells was tested by measuring the TOS level. The extracts at all concentrations did not cause any change in the TOS level of U-87 MG cells, while significantly reduced the TOS level of K562 cells. On the other hand, the extract at all concentrations tested did not cause any change in the TOS level on both non-cancerous cells (Figure 3).

APE1 activity was measured to investigate the DNA repair status of *I. graveolens* on cancer and non-cancerous cells. The level of APE1 significantly decreased in a dose-dependent manner on *I. graveolens*-treated U-87 MG cells compared with the control ($p<0.05$ and $p<0.01$). Also, the level of APE1 decreased significantly at all concentrations of the extract on PRCC ($p<0.001$). The level of APE1 in H_2O_2-treated K562 cells was also enhanced compared with the control ($p<0.001$). On the other hand, the level of APE1 decreased at all concentrations of the extract ($p<0.01$). The extracts at concentrations above



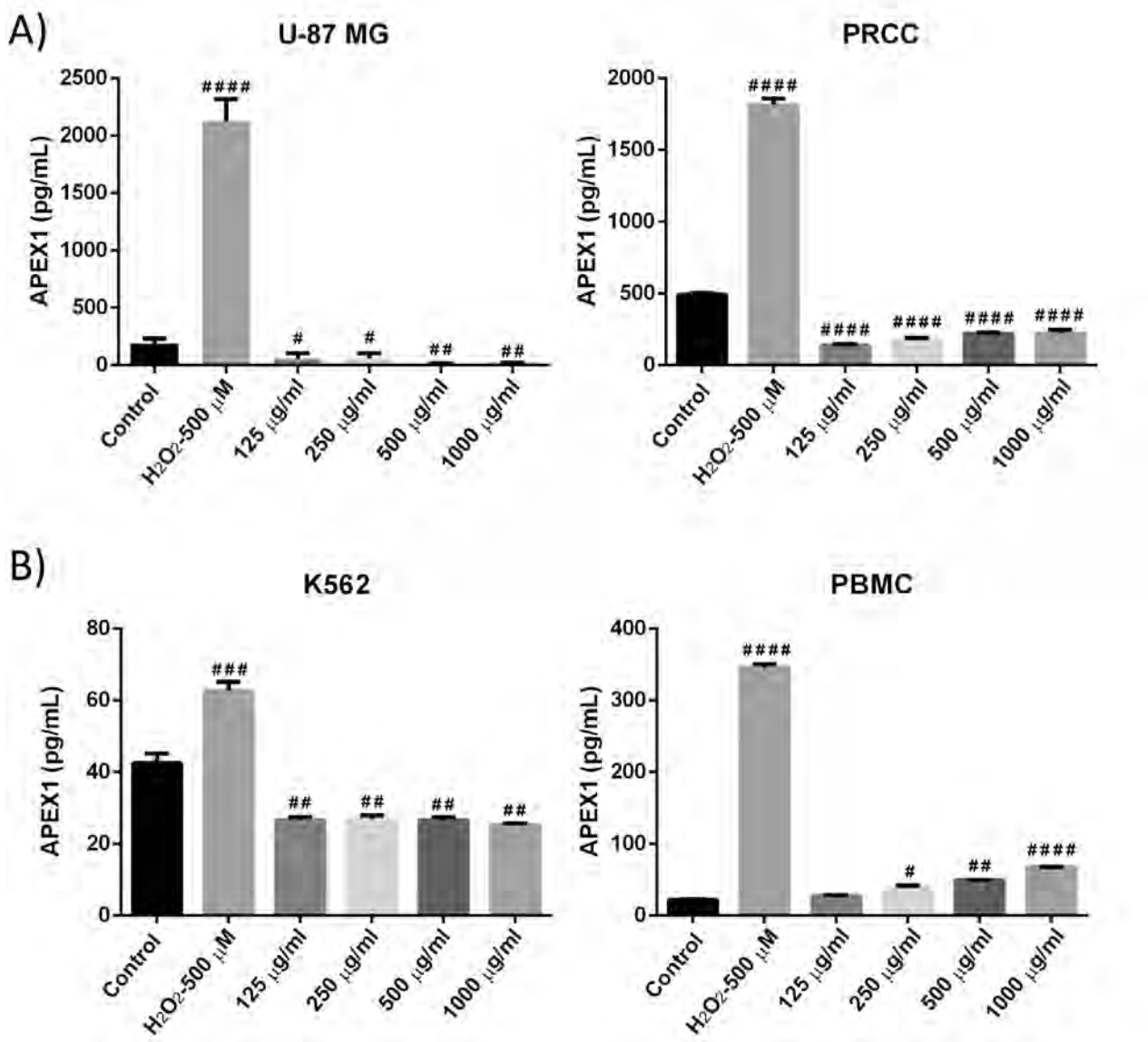
U-87 MG: Human glioblastoma multiforme; PRCC: primary rat cerebral cortex; K562: Chronic myelogenous leukemia; PBMC: peripheral blood mononuclear cells. ##p<0.01, ####p<0.0001 indicate statistically significant differences when compared to nontreated cells or cells treated with other concentrations.

Figure 3. Effects of the *I. graveolens* extract on TOS level in cell lines: (A) human glioblastoma multiforme and primary rat cerebral cortex and (B) Chronic myelogenous leukemia and peripheral blood mononuclear. Values are expressed as mean±standard deviation of three independent replicates.

125 µg/mL led to a dose-dependent increase at the APE1 level in PBMCs (Figure 4).

DISCUSSION

Many of the plants and their products provide numerous opportunities because of their unique properties including easy availability, easy biodegradability, easy handling, low cost, safety for mankind and the environment, greater acceptance among users, and minimum side effects[9]. Plant-derived compounds have been previously shown to suppress tumor onset, development, and progression and may diminish the disease and treatment-related side effects[10]. Similar biological and pharmacological activities of *Inula* species have been demonstrated in previous studies. The anticancer activities of *I. graveolens* were just reported only crude oil extracts, their components, and ethanolic extracts not aqueous parts[4]. Thus, this study aimed to evaluate the antiproliferative potential of aqueous extracts



U-87 MG: Human glioblastoma multiforme; PRCC: primary rat cerebral cortex; K562: Chronic myelogenous leukemia; PBMC: peripheral blood mononuclear cells. #p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001 indicate statistically significant differences when compared to nontreated cells or cells treated with other concentrations.

Figure 4. Effects of the *I. graveolens* extract on apurinic/apyrimidinic endodeoxyribonuclease 1 level in cell lines: (A) Human glioblastoma multiforme and primary rat cerebral cortex and (B) Chronic myelogenous leukemia and peripheral blood mononuclear. Values are expressed as mean±standard deviation of three independent replicates.

of *I. graveolens* on the aggressive solid tumor U-87 MG, liquid malignancy K562 cells, and non-cancerous cells.

Our results clearly showed that extracts of *I. graveolens* exhibited cytotoxic effects toward U-87 MG and K562 cells in a dose-dependent manner. The highest (1000 µg/mL) dose of the extract exerted a highly cytotoxic effect by suppressing the viability of U-87 MG cells 50% without any harmful effects on normal cells. The results of this study are consistent with the previous studies on *I. graveolens*. The crude oil and some pure volatile compounds of *I. graveolens* showed antiproliferative activity on the breast cancer cells[11]. In another study, bornyl acetate and essential oil from *I. graveolens* caused the growth inhibition of HeLa, HT29, A549, MCF7, and FL (human amnion) cells. The essential oil and cisplatin have also exhibited the same inhibitory effect on FL normal cells[12]. *I. graveolens* significantly increased the LDH release in U-87 MG and K562 cells at higher doses. However, the extracts did not affect LDH release on healthy PRCC and PBMCs, except the increased LDH level in PRCC at 1000 µg/mL dose only. It has

been reported that the induction of LDH release in glioma cells had antiproliferative effects. Also, unsaturated fatty acids are high in glia and glioma cell membranes, their peroxidation damages the double-layer structure of the membrane, and this negatively affects important cellular events[13]. Lipid peroxidation-induced cell membrane damages may be associated with the enhanced oxidative stress. TOS is used to determine the oxidative damage and the total response created by antioxidant systems against oxidative stress[14]. *I. graveolens* extracts did not show any statistically significant effect on TOS levels in U-87 MG, PRCC, and PBMC cell lines, while TOS levels decreased in K562 cells. Reactive oxygen species (ROS) plays an important role in the onset and progression of cancer, and although medium ROS levels cause tumor development, excessive levels of ROS suppress tumor progression[15]. The similarity between TOS levels of control and extract-treated glioblastoma cells may be associated with the suppression of excessive oxidant levels. Thus, high oxidative stress is known to cause brain tumor development by damaging cell components[16]. Furthermore, the controversial role of oxidative stress in the suppression of tumor progression may be associated with non-specific oxidative damage to biomolecules in leukemic cells[17]. The results of this study revealed that *I. graveolens* at high concentrations led to apoptosis without causing oxidative stress on cancer cells.

Furthermore, a positive correlation was found between oxidative stress values and APE1 activity on K562 and U-87 MG cells. Oxidative stress in gliomas was shown to increase APE1 activity and, as a result, promote resistance to chemotherapy drugs[18]. In this study, *I. graveolens* significantly reduced the APE1 levels on U-87 MG compared with the control, without increasing oxidative stress. On the other hand, unrepaired abasic sites strongly block DNA replication. Accordingly, the blocking of DNA repair increases cell death in cancer[19]. Thus, the antisense suppression of APE1 as a way of decreasing base excision repair activity enhances the cytotoxicity of chemotherapy in glioma cells[20]. The inverse relationship between cell death and APE1 expression has been established in several studies[21]. It was also demonstrated that glioma cell lines with high APE1 activity also have high radioactive resistance[22]. The decreasing radiation resistance on the cancer cell is more effective in the treatment of glioblastoma that is mandatory to radiotherapy with chemotherapy[23]. Thus, APE1 inhibitors may be a promising new target factor for the treatment strategy of brain cancer[24]. The results of this study revealed that increasing the effectiveness of chemoradiotherapy via inhibition of APE1 provides an advantage for glioblastoma therapy.

CONCLUSIONS

The results of this study first represented that the aqueous extract of *I. graveolens* possesses antiproliferative and cytotoxic activity and also dysfunction in DNA repair mechanism without inducing oxidative stress against human glioblastoma cancer and human leukemic cells *in vitro*. Further comprehensive studies are necessary to identify the active compounds from *I. graveolens* and also to investigate the action mechanism of these compounds on various cancer types.

AUTHORS' CONTRIBUTIONS

KK: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **FA**: Data curation, Formal Analysis, Investigation, Writing – review & editing. **NSO:** Data curation, Formal Analysis, Investigation, Writing – review & editing. **FG:** Project administration. **AT:** Data curation. **OOA:** Data curation. **OC:** Data curation. **GYD:** Data curation.

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ORIGINAL ARTICLE
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Are inflammatory and malnutrition markers associated with metabolic syndrome in patients with sarcoidosis?

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SUMMARY

OBJECTIVE: The study aimed to investigate the use of Neutrophil/lymphocyte ratio, C-reactive protein/albumin ratio, controlling nutritional status, and prognostic nutritional index immune, inflammatory, and malnutrition markers Metabolic syndrome+ in sarcoidosis patients, as an early-stage marker.

METHOD: This is a single-center and cross-sectional study that determines the association of Metabolic syndrome in patients with sarcoidosis. Patients were evaluated based on the National Cholesterol Education Program's Adult Treatment Panel III criteria. Neutrophil/lymphocyte ratio, C-reactive protein/albumin ratio, controlling nutritional status, and prognostic nutritional index values were simultaneously determined through blood test.

RESULTS: A total of 253 patients diagnosed with sarcoidosis were included in this study. Metabolic syndrome– was detected in 37.2% of patients. The prevalence was significantly higher in females (p<0.001). Any degree of malnutrition assessed by controlling nutritional status had higher Metabolic syndrome (p=0.035). The Neutrophil/lymphocyte ratio cutoff value was 2.24, sensitivity was 70.53, specificity was 60.13, and Area Under the Curve value was 0.663 for predicting Metabolic syndrome in sarcoidosis patients.

CONCLUSION: Neutrophil/lymphocyte ratio and controlling nutritional status are associated with the Metabolic syndrome+ in sarcoidosis patients. Thus, close monitoring of Neutrophil/lymphocyte ratio and controlling nutritional status increase in terms of Metabolic syndrome and immune malnutrition may be important in sarcoidosis patients.

KEYWORDS: Metabolic syndrome. Sarcoidosis. Neutrophils. Lymphocytes. Abdominal obesity. C-reactive protein. Albumin. Prognostic nutritional index.

INTRODUCTION

Sarcoidosis is a chronic systemic granulomatous disease that commonly affects the lungs. In the course of this disease, neurological findings, uveitis, blindness, end-stage pulmonary fibrosis, pulmonary hypertension, arrhythmia, cardiomyopathy, hypercalcemia, and renal failure may develop; approximately one-third of these side effects progresses as a chronic disease[1].

Metabolic syndrome (MetS) is a heterogeneous disease that develops on the basis of insulin resistance and involves the combination of systemic disorders such as abdominal obesity,

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glucose intolerance or diabetes mellitus, dyslipidemia, hypertension, and coronary artery disease (CAD)[2]. The neutrophil/lymphocyte ratio (NLR) is a systemic inflammatory marker that can be easily measured and used in the prognosis of several chronic diseases.

In a recent study by Gülhan et al., the coexistence of MetS and insulin resistance was evaluated in patients with sarcoidosis and was found to be increased[3]. Due to MetS components, the risk of early atherosclerosis and the presence of abdominal obesity are particularly important in terms of cardiovascular complications. Our study investigated the predictive value of NLR in predicting the incidence of MetS and the presence of MetS in sarcoidosis patients.

METHODS

The study was designed as an observational, cross-sectional study. The patients who were consecutively admitted as outpatient to the pulmonary medicine department were enrolled. The study was approved by the Local Ethics Committee.

All 345 patients diagnosed with sarcoidosis were screened cross-sectionally to evaluate MetS association and NLR according to the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) criteria. Those who had received or were planning to receive steroid therapy within the past six months, pregnant women, emergency patients, terminal-stage malignancies, and those with active and suspected infectious diseases were excluded from the study. Finally, a total of 253 patients, 94 sarcoidosis with MetS patients and 159 sarcoidosis without MetS patients, were included in the study.

The presence of diabetes mellitus (DM), hypertension (HT), hyperlipidemia (HL), and cardiovascular disease (CVD) was questioned. Based on additional examinations and follow-ups, those who were diagnosed for the first time had DM, HT, HL, and CVD.

Diagnosis of sarcoidosis

Definite diagnosis of sarcoidosis was established through fiber optic bronchoscopy (FOB) in 21.3% (n=53) of patients, endobronchial ultrasonography (EBUS) in 34.1% (n=85) of patients, mediastinoscopy in 38.2% (n=95) of patients, lung biopsy (wedge) in 4% (n=10) of patients, skin biopsy in 2% (n=5) of patients, and lymph node excisional biopsy in the remaining 0.4% (n=1) of patients.

Anthropometric measurements

Each patient underwent a physical examination and a detailed medical examination. Anthropometric measurements and blood pressure measurements were noted. Waist circumference was measured with a tape at the level midway between the lower rib margin and the iliac crest. Blood pressure was measured in the sitting position using a mercury sphygmomanometer with the patients' arm at the level of the heart after they had rested for 15 min in the outpatient clinic.

All patients were evaluated for MetS according to the NCEP-ATP III criteria[4]. The presence of at least three of the five factors defined by ATP III for MetS was accepted as a diagnosis of MetS. European criteria (male ≥94 cm; female ≥80 cm) were used for waist circumference measurement.

Biochemical analysis

Blood samples were collected after 12 h of fasting. Fasting blood glucose, cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, HbA1C, insulin, angiotensin-converting enzyme (ACE), hemogram, albumin, and C-reactive protein (CRP) were analyzed. Neutrophil/lymphocyte and CAR (CRP/albumin ratio) were used for NLR. Prognostic nutritional index (PNI) was calculated as follows: PNI=10×serum albumin (g/dL)+0.005×total lymphocyte count. A value exceeding 2.5 for the homeostasis model assessment of insulin resistance (HOMA-IR) (fasting blood glucose×fasting insulin/22.5) ratio indicates insulin resistance.

Statistical analysis

Variables were investigated using analytical and visual methods (Shapiro–Wilk test and histogram) to determine whether or not they are normally distributed. Continuous variables were presented as mean±SD; if the variables are non-normally distributed, they are presented as median and interquartile range (IQR) 25–75%. Categorical variables were depicted as percentages and numbers. Group comparisons were tested using independent sample t-test or the Mann–Whitney U test, according to distribution of the numerical variables; the chi-square test or the Fisher's exact test was used for the categorical variables. The association between MetS (outcome variable: MetS with sarcoidosis presence) and the CAR, PNI, NLR, age, LDL, and HOMA-IR variables was evaluated using the univariable and multivariable logistic regression models. In addition, receiver operating characteristic (ROC) curve analysis was used to determine whether NLR had discriminative ability for MetS. The independent contribution of each variable to the variance of outcome was estimated. In this regard, the relative importance of each predictor in the model was estimated with a partial 2′ value for each predictor. In addition, correlation analysis was performed for PNI, CAR, HOMA-IR, ACE, and NLR. In all statistical analyses, $p<0.05$ was considered statistically significant. R software version 4.00 (Vienna, Austria) was used for the statistical analysis.

RESULTS

The study population comprised of 253 patients (190 female patients). There was no statistically significant difference between the groups in terms of age, LDL cholesterol, waist circumferences, lymphocyte, insulin, PNI, and ACE. The MetS+ patients had higher neutrophil, CRP, CAR, and NLR than the MetS- patients, other baseline characteristics were described in Table 1.

In sarcoidosis patients, the LDL cholesterol value was 134±33.7 mg/dL, serum triglyceride value was 125 (96–186) mg/dL, HDL cholesterol value was 45±11.2 mg/dL, fasting blood glucose value was 98 (90–190) mg/dL, HbA1c was 6%, waist circumference was 96±11.9 cm, lymphocyte value was 1800 10^3/μL (1400–2300), neutrophil value was 3900 10^3/μL (3100–5000), CRP was 4.2 mg/dL (3.2–7.0), albumin was 4.3 g/L (4.1–4.5), and insulin was 11.4 mIU/L (8.1–17); 28.9% (n=73) of patients had HT, 19% (n=48) had DM, and 49.4% (n=125) had elevated triglyceride levels. Waist circumference was increased in 80.3% of women (n=151) and was statistically significant compared with men (p<0.001). It was increased in 19.7% (n=12) of the male patients, and the median waist circumference was 96 cm; 37.2% (n=94) of patients had MetS. In terms of distribution, 89.3% (n=84) of women and 10.7% (n=10) of men had MetS (p<0.001). The median duration of disease in MetS+ patients with sarcoidosis was found to be four years. Out of 94 MetS+ patients, 57 (60.6%) had HT, and 25 (26.5%) had DM. Out of the 159 MetS- patients, 16 (10%) had HT, and 23 (14.4%) had DM. Fasting blood glucose level was 111 (97–125) mg/dL in MetS+ patients, whereas it was 94 (87–101) in MetS- patients. In MetS+ patients, the neutrophil value was 4700´10^3/μL (3685–5900), CAR was 11.8 (8.01–20.6), NLR was 2.64 (2.09–3.48), and HOMA-IR was 6.73 (3.83–10.2), which were statistically significant (p<0.001).

Binary logistic regression analysis showed that NLR (OR 1.80 [1.21–2.68], p<0.001), LDL (OR 1.49 [1.04–2.15], p=0.003), and HOMA-IR (OR 1.37 [1.04–1.85], p=0.02) were statistically significant compared with MetS-, while the other variables were not (Table 2).

The relative importance of each predictor in the model was presented in Figure 1A; the important variables such as NLR and HOMA-IR were used to predict the presence of MetS in

Table 1. Baseline demographic and clinical variables.

	All (n=253)	MetS– (n=159)	MetS+ (n=94)	p-value
Age (years)	48.6±11.4	49.4±11	47.1±12	0.13
Gender (female %)	190 (75.1)	106 (66.6)	84 (89.3)	<0.001
DM presence (%)	48 (19)	23 (14.4)	25 (26.5)	0.02
HT presence (%)	73 (28.9)	16 (10)	57 (60.6)	<0.001
Glucose (mg/dL)	98 (90–109)	94 (87–101)	111 (97–125)	<0.001
LDL (mg/dL)	134±33.7	133±34.7	139±31.9	0.17
HDL (mg/dL)	45±11.2	49.9±12	43.9±8.5	<0.001
Triglyceride (mg/dL)	125 (96–186)	109 (85.5–138)	179 (141–227)	<0.001
Waist circumference (cm)	96±11.9	96.5±12	97.5±11.7	0.56
Albumin (g/L)	4.3 (4.1–4.5)	4.3 (4.13–4.50)	4.3 (4.10–4.50)	0.46
Lymphocyte (10^3/μL)	1800 (1400–2300)	1800 (1375–2308)	1800 (1400–2200)	0.94
Neutrophil (10^3/μL)	3900 (3100–5000)	3600 (2805–4400)	4700 (3685–5900)	<0.001
CRP (mg/L)	4.2 (3.2–7.0)	3.70(3.20–6.27)	5.20 (3.20–8.74)	0.003
Insulin (mIU/L)	11.4 (8.1–17)	10.9 (8.3–14.5)	13.4 (8.30–18.8)	0.053
CAR	9.76 (7.38–17.8)	8.46 (7.27–13.9)	11.8 (8.01–20.6)	<0.001
NLR	2.25 (1.67–3.15)	2.10 (1.56–2.75)	2.64 (2.09–3.48)	<0.001
PNI	52.2 (49–55.5)	52.0 (49–56)	52.5 (49–55.3)	0.96
HOMA-IR	5.12 (3.69–7.86)	4.76 (3.51–6.42)	6.73 (3.83–10.2)	<0.001
ACE (U/L)	56.8 (37.1–87.5)	58.6 (42.6–87.9)	54.7 (29.1–85.4)	0.29

MetS: Metabolic syndrome; DM: diabetes mellitus; HT: hypertension; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-reactive protein; CAR: C-reactive protein/albumin ratio; NLR: neutrophil/lymphocyte ratio; PNI: prognostic nutritional index; HOMA-IR: homeostatic model assessment-insulin resistance; ACE: angiotensin-converting enzyme. All patients and MetS+ and MetS–.

Table 2. Multivariable logistic regression for predict Metabolic syndrome presence in sarcoidosis.

	Multivariable odds ratio	95%CI	p-value
CRP/albumin ratio (from 7.38–17.76)	1.14	0.99–1.32	0.06
Prognostic nutritional index (from 49–55)	1.03	0.,67–1.56	0.89
Neutrophil/lymphocyte ratio (from 1.66–3.14)	1.80	1.21–2.68	0.003
LDL (from 112–154 mg/dL)	1.49	1.04–2.15	0.03
HOMA-IR (from 3.68–7.85)	1.37	1.04–1.85	0.02
Age (from 40–56 years)	0.77	0.51–1.16	0.22

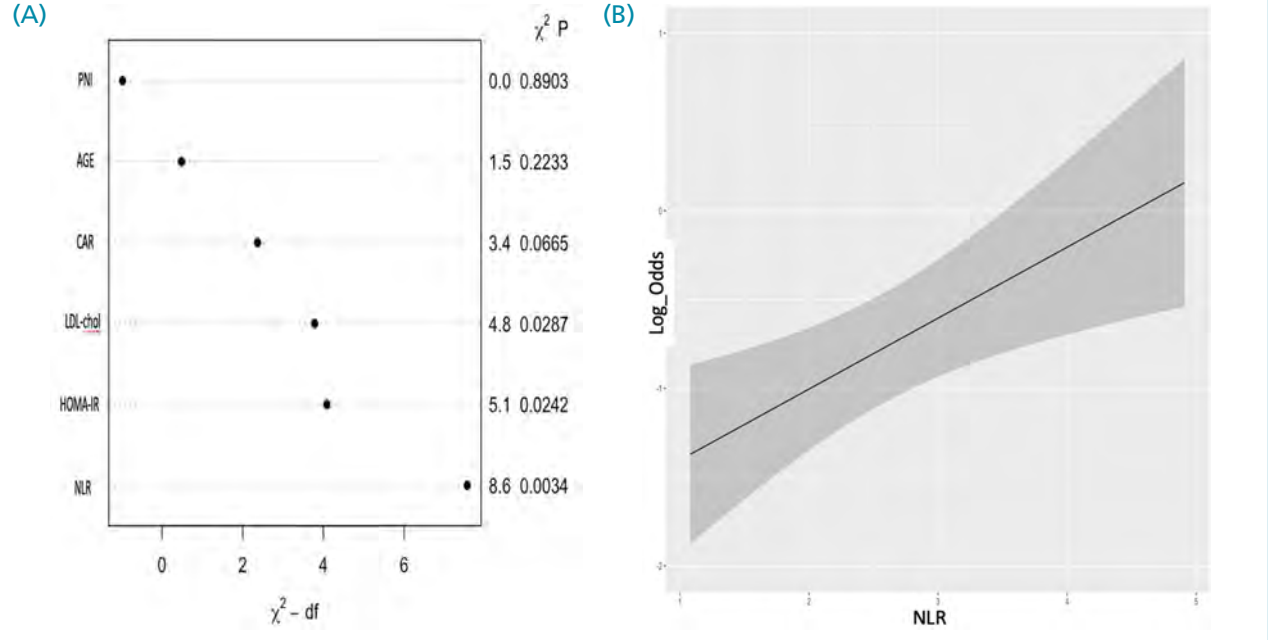
CRP: C-reactive protein; LDL: low-density lipoprotein; CI: Confidence interval; CRP: C-reactive protein; LDL: low-density lipoprotein; HOMA-IR: homeostatic model assessment-insulin resistance. Bold values denote statistical significance at the p<0.05 level.

the sarcoidosis patient. The partial effect plots show the fitted curve on the mean (probability) scale as log-odds (linear predictor) for NLR in Figure 1B.

The NLR cutoff value was 2.24, sensitivity was 70.53, specificity was 60.13, and AUC was 0.663 in predicting MetS in sarcoidosis patients. Herein, a negative correlation existed between NLR and PNI in the correlation analysis [R 0.369 (p<0.001)]. However, there was no correlation between HOMA-IR and NLR, PNI, and CAR.

DISCUSSION

This study showed that NLR was higher in sarcoidosis patients with MetS. Chuan-Chuan Liu et al. evaluated patients in six groups using anthropometric, biochemical, and hematological measurements in terms of MetS marker (NLR) in a study including 34,013 subjects. NLR was concluded to be a good predictor, and the risk increased as this ratio increased. NLR and increased values of NLR could be used as a prognostic marker for the development of MetS[5]. In another study, Kaya et al. investigated the relationship between NLR and CAD using syntax score (SS) in 649 patients with stable angina pectoris and CAD; they determined that NLR was a measurable



PNI: prognostic nutritional index; age: years; CAR: C-reactive protein/albumin ratio; LDL: low density lipoprotein; HOMA-IR: homeostatic model assessment–insulin resistance; NLR: neutrophil/lymphocyte ratio.

Figure 1. **(A)** Relative importance of each variable in the multivariable model for predict presence of metabolic syndrome in sarcoidosis. **(B)** Partial effect plot of neutrophil/lymphocyte ratio for predicting presence of metabolic syndrome+.

systemic inflammatory marker. In multivariate analysis, NLR was associated with the presence and severity of CAD[6].

In their studies including 1300 sarcoidosis patients, Güngör et al. investigated the use of NLR as a marker of inflammation in sarcoidosis. It was concluded that NLR could be used as an inflammation marker, and studies with large patient populations were needed for activity and staging in prognosis[7].

In the report of Balta et al., NLR value was suggested as an independent prognostic factor for CAD, which may be affected by vascular disease-associated MetS, DM, HT, and hypercholesterolemia[8]. In their case-control study, Büyükkaya et al. divided MetS+ patients into three groups (based on their components). MetS+ patients had significantly higher NLR values compared with the control group, and it was observed that the NLR increased with increasing severity ($r=0.586$, $p<0.001$)[9]. Similarly, as a result of our study, NLR was statistically significant as predicted by MetS+. In addition, in the study conducted by Bahadır et al, correlation analysis was performed by comparing metabolic and inflammatory markers between the groups, and it was concluded that NLR is not a good marker of inflammation, and leukocyte and hs-CRP values may be more useful biomarkers to indicate inflammation in nondiabetic patients with obesity and MetS[10].

In our study, 37.15% MetS+ and 6.73 (3.83–10.2) HOMA-IR values were higher in women and were statistically significant ($p<0.001$). In the study conducted by Cozier et al., the relationship of obesity and weight gain with the incidence of sarcoidosis was evaluated in 59,000 US black women aged between 21 and 69 years; of these, the development of sarcoidosis was reported in 454 patients during a 16-year follow-up period (1995–2011). The incidence of sarcoidosis increased with increasing body mass index and weight gain[11].

In the study conducted by Moon et al., patients with elevated CAR and DM were at higher risk of all-cause mortality compared with those without elevated CAR and DM[12]. Similarly, significant results were achieved with CAR and NLR values in predicting inflammation in MetS+ patients ($p<0.001$).

Gvozdenovic et al. conducted a case-control study with 184 patients and evaluated the effect of high body mass index (BMI) on patient-reported results in sarcoidosis patients and healthy individuals, and the highest risk (more than three times) was detected in obese women[13]. In this study, MetS+ was more common in women and was statistically significant ($p<0.001$). In the recently published review, the importance of inflammatory parameters was stated, but malnutrition was left out[14]. According to the results of our research, sarcoidosis patients may need to have their inflammation and malnutrition assessed.

Limitations

This study has some limitations. Being a single-center study and its observational nature is one of the limitations of our study. Our findings should be confirmed in prospective and large-scale studies involving other inflammatory biomarkers to clarify the exact mechanistic role of NLR in sarcoidosis with MetS+.

CONCLUSIONS

In addition to classical parameters, NLR can be used in sarcoidosis patients to predict MetS+. The use of NLR, a strong inflammation marker, may be considered for the closer follow-up needed in patients with MetS+ sarcoidosis. Sarcoidosis patients should be followed up closely in terms of possible comorbidities through separate evaluation in terms of MetS components in their long-term follow-up.

AUTHORS' CONTRIBUTIONS

ACI: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. **MK:** Conceptualization, Data curation, Writing – original draft. **SB:** Data curation, Visualization. **AK:** Formal Analysis, Methodology, Visualization, Writing – original draft. **GK:** Formal Analysis, Investigation, Methodology. **NŞ:** Investigation, Methodology, Visualization.

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Are patients with obesity "scapegoats"? The obesity prejudice levels of health care workers in Turkey

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SUMMARY

OBJECTIVE: This study assessed obesity prejudice levels, attitudes, and perceptions of health care workers toward individuals with obesity and the relationship between health care workers' perceptions of own and society's weight bias, healthy lifestyle preferences, body mass index, and other factors with obesity prejudice levels.

METHODS: This cross-sectional and descriptive study was conducted with 700 health care workers in Turkey via an online survey addressing characteristics, perceptions, and attitudes toward obesity including an obesity prejudice scale. Categorical variables were expressed as frequencies and percentages. The χ^2 test was applied to compare categorical variables. The distribution of the data was evaluated by the Kolmogorov-Smirnov test. Normally distributed data were compared by an independent sample *t*-test, while the Mann-Whitney U and Kruskal-Wallis tests were used for comparing non-normally distributed data.

RESULTS: Participants' mean age was 40.2±11.3 years and 67.9% were women. Notably, 57.9% worked at tertiary health care centers, 85.9% were physicians, and 64.8% were family physicians; 25% were prejudicial, while 58.1% tended to have prejudice toward individuals with obesity. Obesity prejudice scores were significantly higher among those who were in close contact with and who stated their preference for patients with obesity.

CONCLUSIONS: Half of the participants tended to have prejudice, and one-fourth were prejudicial toward individuals with obesity. These results highlight the necessity of raising awareness of health care workers to reduce prejudicial attitudes that may negatively impact patients with obesity. Stigmatizing experiences might be detrimental, reducing the quality of life with long-term consequences for emotional and physical health.

KEYWORDS: Obesity. Weight prejudices. Obesity bias. Stigmatization.

INTRODUCTION

Obesity is a common health problem and individuals impacted by this epidemic have to struggle with concomitant disorders including low self-esteem and depression[1]. Despite improvements in the management of obesity, due to multifactorial etiologies including genetic, environmental, sociocultural, and psychological factors, prevention and treatment should be based on a biopsychosocial and patient-centered approach rather than a biomedical approach[2,3].

Attitudes of health care workers (HCWs) while counseling and managing the patients with obesity are crucial. Reports show that discriminative and stigmatized behavior decreases quality of life, leads to social isolation, decreases applications to health institutions, and affects individual health and society due to an increase in obesity-related problems[4]. Previous studies showed that the percentage of HCWs with prejudice toward patients with obesity was higher than estimated[4,5]. Reports show that physicians had negative feelings about patients with obesity and

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described them as "irresponsible," "incompatible with the management," or "disobeying." Moreover, they tend to spend less time with such patients and demand more laboratory work[4-6].

In our country, studies that measure obesity prejudice levels of HCWs are limited and include only primary care physicians' and medical/nursing students' attitudes and discriminative behaviors toward patients with obesity. This study aimed to investigate the perceptions, attitudes, and opinions of HCWs in Turkey toward individuals with obesity and evaluate the relation between the obesity prejudice levels and sociodemographic characteristics, body mass index (BMI), lifestyle preferences, and other factors.

METHODS

This cross-sectional study was conducted between February and March 2021 via an online questionnaire sent to HCWs working in Turkey.

The sample size was calculated as 385 participants by using the formula, OpenEpi, version 3: $n=[DEFF*Np(1-p)]/[(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$ by accepting the total number of HCWs in Turkey as (N) 1.033.767, the percentage of obesity prejudice possibility existence (p) as 50%, confidence interval as 95%, and confidence limit (d) as 5%.

The online questionnaire form was sent to HCWs as a Google form via Whatsapp, Facebook, and email groups. The participants were first informed about the purpose of the study, the duration of the questionnaire, the identity of the researchers, and how the data would be kept. Participants who accepted to enter the study and responded positively to the consent form then completed the questionnaire.

The questionnaire was developed based on the revision of related articles, then tested on 15 HCWs, and reorganized for accuracy and clarity[4,7]. The self-administered questionnaire form included 53 questions to be completed in 10 min. The questionnaire consisted of two parts. The first part included questions about participants' sociodemographic characteristics, exercise, healthy eating status, body weight and height, attitudes, and perceptions regarding obesity. The second part included questions from the Obesity Prejudice Scale (GAMS-27).

Obesity prejudice scale

The scale was developed by Ercan et al. and included 27 items[8]. Twelve items included positive expressions, and 15 items included negative expressions, defined as "absolutely agree", "agree", "doubtful", "disagree" and "absolutely disagree" as fivefold Likert scale. Negative expressions were evaluated by reverse scoring. The highest and lowest scores would be 135 and 27, respectively. A score of 68.00 and below was evaluated as unbiased, between 68.01 and 84.99 points tend to be biased, and above 85 points was evaluated as biased[8].

Statistical analysis

Data were evaluated by SPSS 21.0. Continuous variables were presented as mean±standard deviation or median and minimum–maximum. Categorical variables were expressed as frequencies and percentages. The chi-square test was applied to compare categorical variables. The distribution of the data was evaluated by the Kolmogorov-Smirnov test. Normally distributed data were compared by an independent sample t-test, while the Mann-Whitney U and Kruskal-Wallis tests were used for comparing non-normally distributed data. BMI of the participants was calculated from the data on SPSS, using the formula (the body weight in kilograms divided by body height in meters squared). The study was approved by Tepecik Research and Training Hospital Ethics Committee (dated January 25, 2021, decision number: 2021/01-57).

RESULTS

The study included 700 Turkish HCWs. Participants' mean age was 40.2±11.3 years, 67.9% were women, and 66.9% were married. Notably, 57.9% of participants were working at tertiary health care centers, 85.9% were physicians, 64.8% were family physicians, and 41.6% worked for less than 10 years. According to calculated BMI values, 15.4% had obesity, 25% were prejudicial, and 58.1% showed prejudice toward individuals with obesity (Table 1). Notably, 29.1% considered losing weight as "difficult" or "very difficult," 79% thought that society prefers thin individuals than those with obesity, 83% had relatives with obesity, 42.3% experienced obesity in their lifetime, 70.4% went on a diet previously, and 77.9% stated difficulty with dieting. In addition, 84.4% were exercising, 48.4% had healthy eating habits, and 81.7% of the participants were in close contact with individuals with obesity. Also, 15.1% were fond of providing health care to patients with obesity, and 3.4% preferred to have individuals with obesity to be around; 72.7% thought that obesity imposes a tremendous economic burden on the health system (Table 2).

Table 1. Categorization of Study Participants' Obesity Prejudice Scale Scores.

Obesity Prejudice Scale Scores	n (%)
Non-prejudicial	118 (16.9)
Tendency for prejudice	407 (58.1)
Prejudicial	175 (25.0)

Table 2. Characteristics of the study participants.

Characteristics and perceptions of HCWs	Category	n (%)
Sex	Male	225 (32.1)
	Female	475 (67.9)
Marital status	Married	468 (66.9)
	Single	232 (33.1)
Duration of work	<10 years	291 (41.6)
	10–20 years	156 (22.3)
	>20 years	253 (36.1)
Medical Workplace	Primary care	189 (27.0)
	Secondary care	106 (15.1)
	Tertiary care	405 (57.9)
Occupation	Non-physician	100 (14.3)
	Physician	600 (85.7)
Medical Specialty of Physicians	Family physician	389 (64.8)
	Other than the family physician	211 (35.2)
BMI	Normal	359 (51.3)
	Overweight	233 (33.3)
	Obesity	108 (15.4)
Self-assessment of own body weight	Normal	353 (50.4)
	Overweight	287 (41.0)
	Obesity	60 (8.6)
Having difficulty in losing weight	Very difficult-Difficult	204 (29.1)
	Do not know	320 (45.7)
	Very easy-Easy	176 (25.1)
Thinking that society prefers thin individuals to fat ones	Agree	553 (79.0)
	Doubtful	88 (12.6)
	Disagree	59 (8.4)
Presence of relative with obesity	Yes	581 (83.0)
	No	119 (17.0)
Presence of own obesity in a lifetime period	Yes	296 (42.3)
	No	404 (57.7)
Doing exercise	Yes	591 (84.4)
	No	109 (15.6)
Eating habits	Very healthy	339 (48.4)
	Healthy	192 (27.4)
	Unhealthy	169 (24.1)
Experience of dieting	Yes	493 (70.4)
	No	207 (29.6)
Having difficulty with dieting	Yes	384 (77.9)
	No	109 (22.1)

Continue...

Table 2. Continuation.

Characteristics and perceptions of HCWs	Category	n (%)
Having prejudice towards individuals with obesity	Yes	221 (31.6)
	No	479 (68.4)
Being in close contact with patients with obesity	Yes	572 (81.7)
	No	128 (18.3)
Preferring individuals with obesity to be around	Yes	24 (3.4)
	Doubtful	493 (70.4)
	No	183 (26.1)
Fond of providing health care to patients with obesity	Yes	106 (15.1)
	Neutral	401 (57.3)
	No	193 (27.6)
Thinking that patients with obesity impose a tremendous economic burden on the health system	Yes	509 (72.7)
	No	191 (27.3)

According to Obesity Prejudice Scale (GAMS-27), female HCWs (p=0.006), married HCWs (p=0.008), and those who worked more than 20 years (p<0.001) were found to be prejudicial compared with males, with those unmarried, and with the participants who worked less than 20 years, respectively. The study participants who stated not to have prejudice toward individuals with obesity (p<0.001), who preferred to have individuals with obesity around (p<0.001), who stated their interest in providing health care to the patients with obesity (p<0.001), and the respondents who did not think patients with obesity cause a great burden to the health system (p<0.001) had significantly higher scores and were found to be prejudicial. The obesity prejudice scale scores did not differ regarding the study participants' workplace, medical specialty, BMI, attitude about society's preference for thin or fat persons, having a relative with obesity, the experience of obesity, doing exercise, healthy eating, the experience of dieting, or having difficulty in dieting (Table 3).

DISCUSSION

- Obesity management is complex. Stigmatization, bias levels, and HCWs' attitudes have significant roles on the effectiveness of communication with patients with obesity.
- As HCWs are in the frontline of managing obesity, the awareness of their own weight bias is important in reducing prejudice.
- The present study was carried out with the largest sample of HCWs in Turkey and contributes to valuable data.

Obesity prejudice among HCWs leads to extra social, psychological, and medical problems. Motivational interviewing techniques are needed for improving the adherence to treatment in obesity[9]. It is important to approach in an empathic, supportive, explanatory, realistic, and guiding way in the patient-centered and motivational interviewing model because patients avoid health care services due to negative attitudes, behaviors, and suggestions for losing weight when the patients are not ready, and failure of past treatments[5-7]. Studies have shown that physicians did not prefer to treat patients with obesity and stated that they did not expect the patients to be successful in losing weight due to nonadherence to treatment[10]. In our study, 31.6% of HCWs were found to have prejudice toward individuals with obesity. Akman et al. reported weight bias among physicians and nurses working at primary care but did not assess by an obesity prejudice scale[7]. A study conducted with nursing students in Turkey reported prejudice scale scores as 75.54, showing that participants were prone to weight bias, and previous studies with students from health-related departments demonstrated that participants had negative attitudes and prejudice toward individuals with obesity. This suggests that studying in health-related departments may not be enough to eliminate or prevent weight bias. Our study found that obesity prejudice levels increased with the duration of work, and a study in Turkey also found that registered nurses had more negative prejudice scores toward persons with obesity than those of student nurses[11].

In our study, HCWs with obesity did not consider themselves as having obesity but overweight (6.8 out of 15.4%) instead. Moreover, study participants with obesity had higher prejudice scale scores than others, but this was not significant.

Table 3. Comparison of Obesity Prejudice Scale categories by study participants' characteristics.

Characteristics	Category	Non-prejudicial	Tendency for having prejudice	Prejudicial	p
		n (%)	n (%)	n (%)	
Sex	Male	35 (15.6)	149 (66.2)	41 (18.2)	**0.006**
	Female	83 (17.5)	258 (54.3)	134 (28.2)	
Marital status	Married	67 (14.3)	271 (57.9)	130 (27.8)	**0.008**
	Single	51 (22.0)	136 (58.6)	45 (19.4)	
Duration of work	<10 years	66 (22.7)	172 (59.1)	53 (18.2)	**<0.001**
	10-20 years	28 (17.9)	84 (53.8)	44 (28.2)	
	>20 years	24 (9.5)	151 (59.7)	78 (30.8)	
Workplace	Primary care	28 (14.8)	116 (61.4)	45 (23.8)	0.189
	Secondary care	12 (11.3)	61 (57.5)	33 (31.1)	
	Tertiary care	78 (19.3)	230 (56.8)	97 (24.0)	
Occupation	Non-physician	20 (20.0)	46 (46.0)	34 (34.0)	**0.024**
	Physician	98 (16.3)	361 (60.2)	141 (23.5)	
Medical specialty of physicians	Family Physician(FP)	73 (18.8)	231 (59.4)	85 (21.9)	0.067
	Other than the FP	25 (11.8)	130 (61.6)	56 (26.5)	
BMI	Normal weight	69 (19.2)	203 (56.5)	87 (24.2)	0.082
	Overweight	33 (14.2)	148 (63.5)	52 (22.3)	
	Obesity	16 (14.8)	56 (51.9)	36 (33.3)	
Self-assessment of own body weight	Overweight	42 (14.6)	172 (59.9)	73 (25.4)	0.201
	Normal weight	65 (18.4)	207 (58.6)	81 (22.9)	
	Obesity	11 (18.3)	28 (46.7)	21 (35.0)	
Having difficulty in losing weight	Very difficult- Difficult	42 (20.6)	108 (52.9)	54 (26.5)	**0.048**
	Do not know	43 (13.4)	189 (59.1)	88 (27.5)	
	Very easy-Easy	33 (18.8)	110 (62.5)	33 (18.8)	
Thinking that society prefers thin individuals to fat ones	Neutral	10 (11.4)	53 (60.2)	25 (28.4)	0.457
	Agree	100 (18.1)	316 (57.1)	137 (24.8)	
	Disagree	8 (13.6)	38 (64.4)	13 (22.0)	
Presence of relative with obesity	Yes	91 (15.7)	345 (59.4)	145 (25.0)	0.15
	No	27 (22.7)	62 (52.1)	30 (25.2)	
Presence of own obesity in a lifetime period	Yes	48 (16.2)	173 (58.4)	75 (25.3)	0.925
	No	70 (17.3)	234 (57.9)	100 (24.8)	
Doing exercise	Yes	99 (16.8)	342 (57.9)	150 (25.4)	0.863
	No	19 (17.4)	65 (59.6)	25 (22.9)	
Eating habits	Healthy as possible	52 (15.3)	204 (60.2)	83 (24.5)	0.371
	Healthy	30 (15.6)	115 (59.9)	47 (24.5)	
	Unhealthy	36 (21.3)	88 (52.1)	45 (26.6)	
Experience of dieting	Yes	84 (17.0)	284 (57.6)	125 (25.4)	0.905
	No	34 (16.4)	123 (59.4)	50 (24.2)	

Continue...

Table 3. Continuation.

Characteristics	Category	Non-prejudicial	Tendency for having prejudice	Prejudicial	p
		n (%)	n (%)	n (%)	
Having difficulty with dieting	Yes	66 (17.2)	216 (56.3)	102 (26.6)	0.455
	No	18 (16.5)	68 (62.4)	23 (21.1)	
Having prejudice towards individuals with obesity	Yes	52 (23.5)	140 (63.3)	29 (13.1)	**<0.001**
	No	66 (13.8)	267 (55.7)	146 (30.5)	
Being in close contact with patients with obesity	Yes	90 (15.7)	332 (58.0)	150 (26.2)	0.121
	No	28 (21.9)	75 (58.6)	25 (19.5)	
Preferring individuals with obesity to be around	Yes	1 (4.2)	10 (41.7)	13 (54.2)	**<0.001**
	Doubtful	67 (13.6)	292 (59.2)	134 (27.2)	
	No	50 (27.3)	105 (57.4)	28 (15.3)	
Fond of providing health care to patients with obesity	Yes	9 (8.5)	53 (50)	44 (41.5)	**<0.001**
	Neutral	53 (13.2)	246 (61.3)	102 (25.4)	
	No	56 (29)	108 (56)	29 (15)	
Thinking that the patients with obesity impose a tremendous economic burden on the health system	Yes	96 (18.9)	310 (60.9)	103 (20.2)	**<0.001**
	No	22 (11.5)	97 (50.8)	72 (37.7)	

Bold values indicate statistical significance.

Due to psychological disturbance, these participants might first stigmatize themselves, which might cause high prejudice levels. Internalized obesity prejudice is the acceptance of these negative attitudes by individuals with obesity, caused by the stigmatization and prejudice demonstrated by other individuals and resulted in low self-esteem, depression, impaired body image, problems related to body weight, and eating disorders[12]. Similar studies showed that high BMI among HCWs was positively related to prejudice levels[13]. Latner et al. conducted a study among overweight individuals and those with obesity and reported that those with internalized obesity prejudice had more physical, psychological, and social problems and lower quality of life[14]. Our participants who preferred to have individuals with obesity around and interested in providing health care to patients with obesity had higher prejudice scores. Moreover, HCWs who considered themselves without prejudice and did not think obesity as a burden to the health system were found to be prejudicial. In our study, obesity prejudice scale scores did not differ according to the participants having a relative with obesity.

Levels of prejudice differed according to participants' sociodemographic characteristics. Individuals who were male, older, and had family members and friends with obesity were found to have lower hidden anti-obese prejudice. Tsai et al. observed that overweight male participants and those with obesity had a positive attitude toward their body weight compared with female participants[15]. In our study, female HCWs were found to have more prejudice than males. The study participants who were married and worked more than 20 years were also more prejudicial than single ones and those who worked less than 20 years. Obesity prejudice levels increased with the duration of work, possibly the result of challenges after many years with the complexity and difficulty of obesity management. Our study revealed that most HCWs had high prejudice scores, although they stated their preference to provide health care to individuals with obesity, revealing a professional dilemma for HCWs.

Although it was not significant, among the physicians who participated in our study, primary care physicians did not have higher prejudicial scores than those working at secondary or tertiary hospitals. Many of the studies in the literature were performed with primary care physicians, and there are limited studies comparing weight bias levels of physicians from different workplaces. Bocquier et al. reported that 30% of primary care physicians showed negative attitudes toward individuals with obesity[16]. Akman et al. also reported anti-obesity attitudes among primary care workers, including

physicians and nurses, but the study sample was smaller than ours and did not include the attitudes of HCWs working in areas other than primary care[7]. As a specialty, the integrative approach of family medicine is very crucial in complex diseases like obesity. Furthermore, primary care HCWs are very important for counseling and access to health care services. Although similar prejudice levels of primary care physicians are promising in this study, prejudicial attitudes of HCWs, including physicians from any kind of medical workplace, should be considered because all have different roles in the management of obesity.

This study has several limitations. First, the data were collected from individuals through Internet and social media groups. We do not know how the individuals who did not accept to participate in this study differ from these study participants. Second, in this study, we evaluated participants' attitudes, opinions, and perceptions only via their declarations, and we might not have received information about their actual practices.

CONCLUSIONS

- The present study was carried out with the largest sample of HCWs in Turkey and contributes to valuable data on prejudice levels among HCWs.
- One-fourth of participants were found to be prejudicial, and half had a prejudicial tendency.
- Prejudice levels did not significantly differ according to the HCWs' specialty, workplace, or BMI.
- Etiology is multifactorial and individuals with obesity deserve a better attitude from HCWs.

Since weight bias affects the quality of health care provided to individuals with obesity, it is necessary to intervene and develop strategies to reduce or prevent prejudice among HCWs. In light of our findings, HCWs with significant roles in managing obesity should undergo postgraduation education programs that should:

1) Use updated information about the multifactorial etiology of obesity;
2) Raise awareness about the effect of weight bias and stigmatization on doctor–patient relationships; and
3) Have realistic targets in the management of obesity and emphasize the concept of multidisciplinary approaches.

AUTHORS' CONTRIBUTIONS

HP: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **OUT:** Supervision, Writing – original draft, Writing – review & editing. **MKS:** Data curation, Supervision, Writing – original draft, Writing – review & editing. **MD:** Supervision, Writing – review & editing. **HY:** Supervision, Writing – review & editing.

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Mirabegron as effective as oxybutynin for ureteral stent symptoms

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SUMMARY

OBJECTIVE: Ureteral stents usually cause pain and lower urinary tract discomfort. This study aimed to compare the effect of mirabegron with oxybutynin in relieving ureteral stent-related symptoms over time.

METHODS: A prospective, longitudinal, randomized, single-blinded study was conducted. Patients who had a ureteral stent inserted after urolithiasis treatment were classified into two groups and received either oxybutynin 5 mg/day (Group O) or mirabegron 50 mg/day (Group M). The Ureteral Stent Symptoms Questionnaire (USSQ) was applied on the 3rd, 6th, and 15th postoperative days. Group domain scores were compared, and a mixed linear model was used to better assess score differences.

RESULTS: Ureteral Stent Symptoms Questionnaire scores were similar in both groups during all three postoperative days ($p>0.05$). A longitudinal analysis showed that global quality of life and general health improved over time, independently of the use of any of the medications ($p<0.05$), while urinary symptoms and body pain scores were lower over time in participants receiving oxybutynin.

CONCLUSION: Both mirabegron and oxybutynin are equivalent in relieving ureteral stent symptoms. Moreover, some stent symptoms seem to decrease over time despite the use of medication.

KEYWORDS: Ureter. Stents. Ureteroscopy. Kidney calculi.

INTRODUCTION

Ureteral stent-related symptoms (USRS) are a common condition presented in patients undergoing endourological procedures. Lower abdomen and back pain usually occur along with lower urinary tract symptoms.

Alpha blockers are drugs very well studied for this purpose[1-3]. The understanding of their efficacy and low incidence of side effects may be an important factor that warrants the choice by many urologists[2-4]. USRS also recall those of hyperactive bladder, so antimuscarinics, such as oxybutynin, tolterodine, and solifenacin, were also studied[5-8]. Benefits of antimuscarinics are overridden by their potential side effects, such as xerostomy, xerophthalmy, blurred vision, constipation, and flushed skin.

Mirabegron is a beta-3 agonist drug that achieves an effective inhibition of cholinergic pathways without the occurrence of undesired side effects caused by antimuscarinics[9,10].

This study aims to compare the effect of mirabegron with oxybutynin in relieving USRS over time.

METHODS

After project registration and approval by the institutional review board (authorization number 2.552.613/CAAE 85.174.18.0.0000.0103), we performed a prospective, randomized, single-blinded study. From April 2018 to March 2020, 48 patients who underwent urological procedures, in

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which a ureteral stent was inserted, were enrolled. Consenting participants were completely informed regarding benefits and potential risks of using the medication.

Inclusion criteria were to have had a ureteral stent inserted after a minimally invasive urinary stone treatment procedure (either ureteroscopy, percutaneous nephrolithotripsy, or laparoscopy) or simple ureteral stent insertion without definite stone treatment for ureteral calculi. Exclusion criteria comprised patients with a ureteral stent inserted at the time of a previous surgery, pregnant women, forgotten ureteral stents, patients aged below 20 years and above 70 years, patients with known or reported overactive bladder, and men with symptomatic benign prostatic hyperplasia. The ureteral stent used was the same in all cases (Hummer™ Ureteral Drainage Kit 6 Fr x 26 cm).

Participants were randomly classified into two groups through a simple randomization design. The first group (Group O) received, at the day of discharge, oxybutynin 5 mg/day, and the second group (Group M) received mirabegron 50 mg/day for 15 days. They were also prescribed nonsteroid anti-inflammatory drug (etodolac 400 mg twice a day) for additional discomfort.

A Portuguese validated form of the Ureteral Stent Symptoms Questionnaire (USSQ)[11] was given to all participants through a phone call in three different moments (3rd, 6th, and 15th postoperative days). The interviewer was not aware of which medication had been prescribed to the participant. The USSQ design contemplates six separate domains that embrace urinary symptoms, body pain, general health, work performance, sexual matters, and additional problems, as well as an overall global quality of life. In the original validation study, Joshi et al. stated that there is no single score for the whole questionnaire, as individual section scores represent separate domains and characteristics of the stent experience[12]. By acknowledging this assertion, after fully applying the questionnaire, we conducted separate analysis of four domains, namely, urinary symptoms (U), body pain (P), general health (G), and additional problems (A), as well as global quality of life (GQ).

Out of total 48 participants, 24 were initially included in Group O and 24 in group M. Throughout the study, eight participants were excluded due to not completing telephonic interviews. Eventually, the final sample size was 40 (Group O=21, Group M=19).

Statistical analysis was performed using R language (R Core Team, 2017). Numerical variables were represented through mean value and standard deviation. Student's *t*-test was used to compare numerical values at each interview time. Qualitative variables were represented by their absolute and relative frequencies. Fisher's exact test was used to analyze variables at each time of interview. A longitudinal analysis was carried out for each USSQ domain using a mixed linear model with random intercept in order to accommodate initial differences inherent to each participant and induce a correlation structure among observations of a participant in different moments[13]. The number of days after the procedure was used as numerical variable, and Group O was used as reference. For all statistical results, $p<0.05$ was considered significant.

RESULTS

Data related to participants and procedures are given in Table 1. No statistical significance was found between the

Table 1. Participants' characteristics and procedures frequencies.

	Total	Group O	Group M	p-value
Number of participants (n)	40	21	19	–
Gender, n (%)				
Female	27 (67.5)	15 (71.4)	12 (63.2)	0.74
Male	13 (32.5)	6 (28.6)	7 (36.8)	
Age, years (mean±SD)	45.77±10.76	47.9±8.68	43.42±12.49	0.2
Weight, kg (mean±SD)	78.07±15.9	78.8±18.02	76.6±12.26	0.78
Height, cm (mean±SD)	164.8±11.23	165.3±13.02	163.8±7.63	0.78
Procedures				
Cystoscopic double-J insertion, n (%)	14 (35)	8 (38.1)	6 (31.6)	0.84
Laparoscopic pyelolithotomy or ureterolithotomy, n (%)	3 (7.5)	1 (4.8)	2 (10.5)	
Percutaneous nephrolithotripsy, n (%)	1 (2.5)	1 (4.8)	0 (0)	
Ureterolithotripsy, n (%)	22 (55)	11 (52.4)	11 (57.9)	

groups. Frequency of urological procedures between the groups was similar.

Overall results for domain scores at each interview are shown in Table 2. None of the scores showed statistical difference between the groups at any of the interviews.

Random intercept analysis results for all domains are displayed in Table 3. Statistical results for urinary symptoms reveal a significant interaction effect (p=0.03) between the number of days and the group to which the patient belongs, meaning that the effect caused in each group is different for each moment of the analysis. As Group O is the reference for this model, the intercept value can be interpreted as the estimate value for urinary symptoms for Group O at time zero. Group effect, in this case, varies according to the number of days.

Analysis of the body pain domain shows significant interactions for both time (p<0.05) and Group (p=0.02), indicating that both time and medication may play a role in the participant's symptoms, benefiting patients in Group O. General health analysis shows a significant time effect (p<0.05), with similar decrease in both groups. Global quality of life reveals a significant time effect for both groups (p<0.05) but no difference between them.

DISCUSSION

The use of antimuscarinics (oxybutynin and tolterodine) has been widely studied, with data showing benefit when used

Table 3. Linear mixed model with random intercept analysis of the domains.

Urinary symptoms	Estimative	p-value
Group M compared with Group O	-1.49	0.51
Days after procedure	-0.15	0.21
Group M interaction: Days	0.40	0.03*
Body pain	**Estimative**	**p-value**
Group M compared with Group O	-2.72	0.40
Days after procedure	-0.71	0.00*
Group M interaction: Days	0.41	0.02*
General health	**Estimative**	**p-value**
Group M compared with Group O	0.26	0.89
Days after procedure	-0.26	0.00*
Group M interaction: Days	0.07	0.41
Additional problems	**Estimative**	**p-value**
Group M compared with Group O	0.84	0.21
Days after procedure	-0.04	0.10
Group M interaction: Days	0.02	0.55
Global quality of life	**Estimative**	**p-value**
Group M compared with Group O	-0.33	0.42
Days after procedure	-0.07	0.00*
Group M interaction: Days	0.03	0.15

*Statistically significant.

Table 2. Domains' scores (mean±SD) at different times of interview.

3rd postoperative day	Group O	Group M	p-value
Urinary symptoms	29.62±5.08	28.95±6.84	0.72
Body pain	24.38±13.25	22.79±9.16	0.66
General health	14.38±6.09	14.95±7.34	0.79
Additional problems	5.90±1.89	6.84±2.22	0.16
Global quality of life	4.76±1.30	4.53±1.39	0.58
6th postoperative day	**Group O**	**Group M**	**p-value**
Urinary symptoms	26.52±4.93	27.89±6.46	0.45
Body pain	20.1±9.91	20±9.14	0.97
General health	12.57±5.60	13.11±6.38	0.78
Additional problems	5.43±1.57	6.37±2.11	0.12
Global quality of life	4.09±1.09	4±1.20	0.79
15th postoperative day	**Group O**	**Group M**	**p-value**
Urinary symptoms	27±7.52	31.32±8.82	0.11
Body pain	15.14±8.37	18.58±9.02	0.22
General health	10.9±4.84	12.21±6.30	0.47
Additional problems	5.29±2.08	6.47±2.36	0.1
Global quality of life	3.75±1.41	3.95±0.91	0.6

SD: standard deviation.

alone or in association with alpha blockers (alfuzosin and tamsulosin)[6,7]. Mirabegron, a beta-3 agonist approved for OAB symptoms, has also been proved to perform a selective alpha-1a and alpha-1d adrenergic antagonism[14] in an experimental scenario. In addition, Shen et al.[15] recently demonstrated, through immunochemistry analysis, the expression of all beta-adrenergic receptor subtypes in the mucosa and muscular layers of the human ureter. These findings reinforce a theoretical benefit for mirabegron to relief USRS.

Results shown in Table 2 support mirabegron to be as effective as oxybutynin in relieving USRS. Similarly, Tae et al. first reported that mirabegron markedly reduced body pain when compared with placebo (21.96 *versus* 13.96, p=0.007) but did not observe significant differences in the other domains[16]. In this study, a subanalysis of the urinary symptoms' domain revealed a significant improvement only in specific scores that were mainly related to storage symptoms[16]. Yavuz et al. performed a prospective placebo-controlled study comparing tamsulosin and mirabegron with a lesser use of analgesics by patients in the mirabegron group, although no reduction was seen in urinary symptoms scores when compared with placebo[17]. Finally, Cinar et al. described a significant reduction in USRS with the use of mirabegron as a monotherapy in a retrospective study[18]. Therefore, this is the first study that compares mirabegron with an antimuscarinic for USRS.

This study provides an in-depth understanding about ureteral stent symptoms over time. According to Liu et al., in a study that compared tamsulosin, solifenacin, and their combination with placebo, multiple interviews were performed over time and the authors concluded that USRS decreased spontaneously in all groups, including placebo, within the four initial days[19]. In our study, with the mixed linear model with random intercept, we could understand how domains' scores behave over time despite the use of the drugs without the need for a placebo group. Within the body pain domain, time played a role in easing the symptoms (p<0.05). For this domain specifically, there is also a difference between the groups. There was less reduction of pain during time in Group M than in Group O (p<0.02). However, this finding does not impact the overall score comparison at different interview dates. Regarding urinary symptoms, there is also a reduction of the score over time, but this reduction was significantly important in Group O alone (p=0.03). This finding coincides with that suggested by Tae et al.[16] and Yavuz et al.[17], whose studies indicate that mirabegron did not show improvement in urinary symptoms when compared with placebo. Again, the differences in the overall score on interview dates did not show statistical significance. Time effect could be demonstrated in general health (p<0.05) and global quality of life (p<0.05) domains.

The small number of patients in each group is a limitation of this study. Our institution suffered important interruption of the surgical routine due to the coronavirus disease 2019 pandemic, which significantly impacted patient enrollment and data collection. We are also aware that this study has the limitation of not having better homogenized groups regarding the type of procedure. Nevertheless, our sample comprehend an excerpt of patients with ureteral stents that represent a reality not far from other services and practices. We consider this study an important contribution to the efforts in finding a better medication to ease USRS in real practice.

CONCLUSION

Mirabegron and oxybutynin are equivalent in relieving ureteral stent symptoms. We also found evidence supporting the hypothesis that some stent symptoms simply decrease over time, independently of medication. Further studies are needed to complement our findings.

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AUTHORS' CONTRIBUTIONS

PJ: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **GFM:** Conceptualization, Data curation. **GMF:** Data curation, Project administration. **MHO:** Formal Analysis, Writing – original draft. **RF:** Conceptualization, Supervision, Writing – review & editing.

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Relationship between pressure and thermal pain threshold, pain intensity, catastrophizing, disability, and skin temperature over myofascial trigger point in individuals with neck pain

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SUMMARY

OBJECTIVE: The objective of the study was to correlate the thermal pain threshold (heat and cold) on myofascial trigger points with measurements of pain and skin temperature in patients with chronic neck pain.

METHODS: This is a cross-sectional study. We included participants of both genders, aged between 18–45 years, with chronic neck pain (>90 days), and with active bilateral myofascial trigger point centrally located in the upper trapezius muscle. Neck Disability Index, Numerical Rating Scale, Pain-Related Catastrophizing Thoughts Scale, algometry, infrared thermography, and quantitative sensory testing were used for the evaluation.

RESULTS: A significant, weak, and negative association was observed between pain intensity and heat pain threshold on the myofascial trigger point to the right (rho -0.381, p=0.022) and to the left (rho -0.334, p=0.049), and a significant, weak, and positive association was observed between pain intensity and cold pain threshold on the myofascial trigger point to the right (rho 0.471, p=0.004) and to the left (rho 0.339, p=0.043).

CONCLUSION: Thermal pain threshold (heat and cold) on myofascial trigger points is associated with pain intensity in individuals with chronic neck pain.

KEYWORDS: Physical therapy. Myofascial pain syndrome. Muscle.

INTRODUCTION

Myofascial trigger points are dysfunctional structures present in several primarily musculoskeletal diseases, such as neck pain[1]. Considering the multidimensional characteristic of pain, the assessment of a patient with myofascial trigger points also involves the measurement of aspects related to peripheral and central sensitization[2]. The quantitative sensory testing (QST) measures the somatosensory function and can be used as an instrument to investigate inadequate function (hypoalgesia) as well as gain of function (hyperalgesia). In addition, QST

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allows the assessment of allodynia, an important marker of central sensitization[3-5].

The scientific literature is scarce in terms of studies that investigated the thermal pain threshold on myofascial trigger points. This clinical measure is important to identify the functioning of peripheral reception and interpretation of hot and cold stimuli[6,7]. Thus, describing these aspects is important to understand the clinical complexity involved in chronic painful disorders associated with the presence of myofascial trigger points.

Given the above, this study aimed to correlate the thermal pain threshold (heat and cold) on myofascial trigger points with measurements of pain and skin temperature in patients with chronic neck pain.

METHODS

Ethical aspects

The research procedures were approved by the Research Ethics Committee of the institution, opinion number 030643/2013. The recruitment of volunteers took place in communities in the city of Ribeirão Preto (SP, Brazil). The invitation to participate was through verbal communication, posters, radio, and the Internet.

Study design

This is a cross-sectional study, in which a physiotherapist was responsible for the recruitment, diagnosis of neck pain and myofascial trigger points, assessment of pain and skin temperature, a second professional was responsible for the assessment of the thermal pain threshold, while a third physiotherapist processed and analyzed the collected data.

Sample

The processing of the sample calculation was performed using the software Ene, version 3.0 (Universitat Autònoma de Barcelona, Barcelona, Spain). The sample size was calculated based on a previous study[8]. The calculation was based on the detection of moderate association (r 0.50) between the variables. Thus, considering a statistical power of 90% and alpha of 0.05, a total of 34 volunteers were estimated.

Inclusion criteria were as follows: participants of both genders, aged between 18 and 45 years, and with chronic neck pain (>90 days), which was identified according to the following criteria: Neck Disability Index (NDI) score ≥5 points and Numerical Rating Scale (NRS) score ≥3 at rest or during active cervical movement.

In addition, the volunteers presented an active bilateral myofascial trigger point centrally located in the upper trapezius muscle, diagnosed according to the criteria established by the previous studies[9,10]. It is noteworthy that these diagnostic criteria for myofascial trigger points have acceptable levels of reliability, according to Gerwin et al.[9], with kappa values ranging between 0.36–0.88.

Exclusion criteria were as follows: participants who had a history of cervical trauma; head, face, or cervical surgery; cervical hernia; spinal degenerative diseases; having undergone physical therapy treatment in the past three months; use of analgesics, anti-inflammatory drugs, or muscle relaxants in the previous week; the presence of systemic diseases; medical diagnosis of fibromyalgia; and body mass index (BMI) greater than 28 kg/m^2.

Neck disability index

This is an instrument adapted and validated for the Brazilian population[11], consisting of 10 questions that investigate neck disability and pain. For each question, it is possible to indicate one in six answers, corresponding to scores 0–5. Therefore, the total score ranges from 0–50 points. The higher the score, the greater the disability.

Numerical rating scale

A simple and easy-to-measure scale consisting of a sequence of numbers, from 0 to 10, in which the value 0 represents "no pain" and 10 represents "worst imaginable pain." Thus, volunteers graded their neck pain based on these parameters[12]. Pain intensity was assessed with the individual at rest and after active movements of the cervical spine.

Pain-related catastrophizing thoughts scale

This scale was adapted and validated for the Brazilian population by Sardá Junior et al.[13] to assess pain catastrophizing. The scale is composed of nine items scaled on a Likert scale ranging from 0–5 points associated with the words "almost never" and "almost always." The total score is given by the sum of the items, divided by the number of items answered, with the minimum score being 0 and the maximum 5. There are no cutoff points, with higher scores indicating the greater presence of catastrophic thoughts.

Algometry

An algometer (Instrutherm, model PTR-300, São Paulo, SP, Brazil) was used to measure the pressure pain threshold (PPT). A previously trained examiner positioned the algometer with a rubber disk measuring 1 cm^2 at the end and exerted gradual compression exactly over the myofascial trigger points with a constant velocity of approximately 0.5 kg/cm^2/s, controlled by the sound feedback of a digital metronome[14]. This measurement

was performed bilaterally. These points were pressed until the intensity in which the volunteer reported pain. PPT measurement was performed three times for each muscle and the mean value was considered. This assessment has an inter-rater intraclass correlation coefficient (ICC) value of 0.91[15].

Infrared thermography

To perform this examination, the volunteers remained for 15 min in an environment at a controlled temperature around 22°C. A T300 thermal camera model (FLIR Systems, Wilsonville, OR, USA) with an accuracy of up to 0.05°C was used. We used an emissivity of 0.98. Three infrared images were captured in sequence, at a distance of 100 cm from the volunteer, to allow the framing of the muscles to be evaluated[16].

To determine the temperature value over the myofascial trigger point, the QuickReport software, version 1.2 (FLIR Systems) was used. Skin temperature measurements were based on previous studies, which identified excellent intra- and inter-examiner reliability for punctual analysis of the infrared image on the myofascial trigger point, with ICC values of 0.95 and 0.90, respectively[17]. According to a study by Magalhães et al.[18], the compressive force used to diagnose myofascial trigger points does not affect skin temperature, as long as the procedure is performed 15 min after the application of force.

Quantitative sensory testing

The evaluation of the thermal pain threshold was performed through the QST, using the TSA II Neurosensory Analyzer model equipment (Medoc, Ramat Yishai, Israel) for this purpose. To this end, with the volunteer seated in an air-conditioned environment, the examiner positioned the equipment electrode, bilaterally, on the central myofascial trigger point of the upper trapezius muscle. Three repetitions of the test were performed for the heat stimuli, at an initial temperature of 32°C and a maximum of 50°C, and three repetitions were performed for the cold stimuli, at an initial temperature of 32°C and a minimum of 0°C. The participant was instructed to interrupt the temperature change by pressing a sensor whenever it reached an intensity that caused pain, and this value was recorded. For statistical analysis, the mean of the three repetitions was used.

Statistical analysis

Initially, data distribution was verified using the Shapiro–Wilk test. Therefore, using this observation, the Pearson's correlation coefficient (r) was applied to the correlations between variables with normal distribution and Spearman's (rho) to verify the association between variables with non-normal distribution. To interpret the magnitude of the correlations, a previous classification established[8] was used: weak, from 0.26–0.49; moderate, from 0.50–0.69; high, from 0.70–0.89; and very high, from 0.90–1.00. Data processing was performed using the Statistical Package for Social Sciences software, version 17.0 (Chicago, IL, USA).

RESULTS

A total of 53 volunteers of both genders were recruited for the study and 17 volunteers were excluded for the following reasons: five had an NDI score lower than 5 points; four had pain intensity lower than 3 points according to the NRS; four individuals had latent trigger points; three had unilateral trigger point; and one had no trigger points in the upper trapezius muscle.

Thus, 36 volunteers were included in the study: 33 women, 31 right-handers, mean age of 23.68 years (standard deviation [SD] 4.01), mean BMI of 22.42 (SD 2.93), chronicity mean neck pain 50.91 months (SD 37.87), and mean Beck Depression Inventory score of 5.19 points (SD 2.64). In addition, the values of central tendency and dispersion of the study variables are described in Table 1.

Regarding the correlations between the variables, a significant, weak, and negative association was observed between pain intensity and thermal pain threshold (heat) on myofascial trigger point to the right (rho -0.381, p=0.022) and to the left (rho -0.334, p=0.049), and a significant, weak, and positive association between pain intensity and thermal pain threshold (cold) on myofascial trigger point to the right (rho 0.471, p=0.004) and to the left (rho 0.339, p=0.043) was found. Other details are described in Table 2.

DISCUSSION

Our findings showed that the thermal pain threshold on myofascial trigger points correlates only with pain intensity in chronic neck pain patients. We did not observe a significant correlation with disability, catastrophizing, PPT, and skin temperature. Several studies were carried out with QST and painful disorders. However, our study is the pioneer in investigating the thermal pain threshold in patients with myofascial trigger points.

Considering QST, a systematic review conducted with people with spinal pain identified magnitudes of correlation with pain intensity lower than in the present study (cold pain threshold -0.07, heat pain threshold -0.07), with no clinical importance. Likewise, the authors identified a negligible magnitude of correlation with disability (cold pain threshold -0.22, heat pain threshold -0.02). Thus, the authors concluded that the pain threshold is a poor marker of central sensitization or that sensitization does not play a major role in patients' reporting of pain and disability[19].

Another systematic review carried out with people with musculoskeletal pain noted a weak magnitude of correlation with pain (cold pain threshold 0.14, heat pain threshold -0.14)[20]. A study points out inconsistent results regarding

Table 1. Description of mean values, standard deviation, median, first and third quartile of the study variables.

	Mean	SD	Median	1st quartile	3rd quartile
NRS at rest (score)	3.11	1.75	3.00	2.00	4.00
NRS after movements (score)	5.36	1.79	5.50	4.00	7.00
NDI (score)	11.27	4.15	11.00	8.00	14.00
PCTS (score)	1.29	0.92	1.16	0.55	1.88
Right PPT (kg/cm^2)	1.64	0.42	1.61	1.35	1.91
Left PPT (kg/cm^2)	1.55	0.38	1.46	1.31	1.83
Right ST (°C)	33.24	1.13	33.26	32.31	34.11
Left ST (°C)	33.20	1.20	33.43	32.23	34.01
Right TPT heat (°C)	42.42	3.74	43.11	39.41	45.03
Right TPT cold (°C)	19.73	10.40	24.43	9.09	28.34
Left TPT heat (°C)	42.22	3.69	41.80	38.95	45.26
Left TPT cold (°C)	19.57	9.97	23.40	13.69	28.09

SD: standard deviation; NRS: numerical rating scale; NDI: neck disability index; PCTS: pain-related catastrophizing thoughts scale; PPT: pressure pain threshold; ST: skin temperature; TPT: thermal pain threshold.

Table 2. Correlation between thermal pain threshold and variables related to pain and skin temperature.

	Right TPT heat (°C)	Right TPT cold (°C)	Left TPT heat (°C)	Left TPT cold (°C)
NRS at rest (score)	rho= -0.381, p=0.022*	rho=0.471, p=0.004*	rho=–0.334, p=0.049*	rho=0.339, p=0.043*
NRS after movements (score)	rho= -0.121, p=0.483	rho=0.298, p=0.077	rho=0.043, p=0.802	rho=0.203, p=0.235
NDI (score)	rho= -0.003, p=0.988	rho=0.071, p=0.680	rho=0.036, p=0.834	rho=0.017, p=0.921
PCTS (score)	r= -0.079, p=0.647	rho=0.291, p=0.085	r=0.025, p=0.886	rho=0.191, p=0.265
Right PPT (kg/cm^2)	r=0.105, p=0.541	rho=0.010, p=0.954	r= -0.034, p=0.845	rho=0.119, p=0.448
Left PPT (kg/cm^2)	r=0.091, p=0.600	rho=0.033, p=0.805	r= -0.088, p=0.609	rho=0.178, p=0.298
Right ST (°C)	r= -0.009, p=0.957	rho=0.268, p=0.114	r=0.136, p=0.431	rho=0.116, p=0.499
Left ST (°C)	r=0.038, p=0.827	rho=0.218, p=0.201	r=0.228, p=0.181	rho=0.093, p=0.589

TPT: thermal pain threshold; SD: standard deviation; NRS: numerical rating scale; NDI: neck disability index; PCTS: pain-related catastrophizing thoughts scale; PPT: pressure pain threshold; ST: skin temperature; r: Pearson's correlation coefficient; rho: Spearman's correlation coefficient. *Statistically significant correlation.

the behavior of the thermal pain threshold in patients with migraine[21]. In patients with fibromyalgia, a recent systematic review describes a reduction in cold pain thresholds when compared to a control group[22]. In breast cancer survivors, local disturbance in thermal detection and increased pain facilitation were found in these patients with pain in the surgical area[23].

Other clinical features have already been evaluated in muscles with myofascial trigger points. An important study highlights that the tensiomyography contractile properties did not seem to show differences, while the sonoelastography and mechanosensitivity presented higher stiffness and lower PPT when compared with the control group[24].

Our study has limitations that must be considered. The sample consisted mostly of women. Thus, future studies can investigate the relationship between thermal pain threshold and gender, with greater inclusion of men. In addition, the sample consisted mostly of young people with a less mean age who were involved in most clinical studies on chronic neck pain.

CONCLUSIONS

Thermal pain threshold (heat and cold) on myofascial trigger points is associated with pain intensity in individuals with chronic neck pain.

AUTHORS' CONTRIBUTIONS

AVDF: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – review & editing. **AKO:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **MPO:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **DBG:** Conceptualization, Methodology, Writing – review & editing. **RRJG:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – review & editing.

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Temporal trends in tetanus incidence and lethality in Brazil: analysis of the national database from 2009 to 2018

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SUMMARY

OBJECTIVE: This study aimed to analyze the epidemiological and hospital characteristics of cases and deaths due to accidental tetanus in Brazil.
METHODS: A time-series study with secondary data extracted from the Department of Informatics of the Brazilian Unified Health System. The time series were evaluated by linear regression parameters, considering a significance level of 1%.
RESULTS: A total of 2,772 cases were reported between 2009–2018. Predominant cases were men and aged between 40–59 years old, with incomplete 1st–4th grade. The lethality rate was found to be predominant in women, whites, illiterates, and those who aged above 80 years. The overall lethality was 32.5%. The incidence rate reduced from 1.6 in 2009 to 0.95 per million inhabitants in 2018, but lethality increased from 30.77–40.70%. The highest rate of incidence and lethality occurred in the elderly people and in the northern region.
CONCLUSION: The high cost and lethality of tetanus configure it as a public health problem. The demonstration of the epidemiology of patients who most evolve to death can help to contribute to a reduction in lethality, which shows an increase in the analysis period. Finally, special attention should be given to the elderly people and those living in the northern region.
KEYWORDS: Tetanus. Incidence. Epidemiology. Brazil.

INTRODUCTION

Tetanus is an infectious and noncontagious disease caused by the anaerobic gram-positive bacteria. *Clostridium tetani* can enter the body through a continuity solution caused by injury in the skin or mucosa[1]. Inside the host, the bacterium finds favorable conditions to multiply, take on a filamentous form, and produce neurotoxins[2]. Tetanospamin neurotoxin can act on the nerve terminals, resulting in the failure to inhibit motor reflexes, and generalized contractions of the agonist and antagonist muscles, causing tetanic spasms[3,4].

Treatment includes debridement of focus, antibiotic therapy, immunization, neuromuscular blockers, and early tracheostomy[5]. The high lethality rate and complications of this disease occur mainly due to the dysfunction of the respiratory muscles[6]. All these factors make the treatment of tetanus more complex and expensive for the health system[7].

Despite its acute and severe behavior, tetanus is an immune preventable disease. According to the Basic Vaccination Calendar adopted by the Ministry of Health of Brazil, tetanus immunization, the pentavalent vaccine, should be administered at 2, 4, and 6 months, with boosters at 12–15 months and 4 years as diphtheria-tetanus-pertussis (DTP) vaccine, and between 10–19 years old as diphtheria and tetanus (DT) vaccine, and needs reinforcement every 5 or 10 years if at risk or during

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pregnancy[8]. In addition to scheduled vaccination, an emergency prophylactic dose after exposure to risky situations can reduce by approximately four times the patient's chance of progressing to death[9,10].

In 2020, global tetanus incidence data estimated the occurrence of 11,763 new cases, with the European continent having the lowest incidence and the African continent having the highest incidence, followed by South-East Asia[11]. In Brazil, between 1982 and 2006, tetanus cases reduced by more than 80%, from 1.8–0.22 cases per 100,000 inhabitants. However, the lethality rate did not reduce[12]. This situation is especially worrisome since tetanus is an immune-preventable disease. For this reason, more efficient health measures are desirable, with an approach at the collective level and with interventions at wide population coverage.

Therefore, considering the high lethality of tetanus, the high cost of treating this condition in addition to the scarcity of recent studies that address the epidemiological, hospital, and mortality characteristics of tetanus in Brazil, the objective of the present study was to investigate the epidemiological profile of cases reported and deaths from tetanus, by Brazilian macro-regions from 2009–2018.

METHODS

A time-series study was conducted with secondary data extracted from the Department of Information of the Brazilian Unified Health System (DATASUS). The variables collected included information on the epidemiological profile and hospital data on cases and deaths from tetanus reported in Brazil from 2009–2018. Analyses were carried out at the national geographical levels, the Brazilian macro-regions (North, Northeast, Midwest, Southeast, and South), and the Federative Unit of Brazil.

Secondary data were extracted from the Information System for Notifiable Diseases (SINAN) and the Hospital Information System of the Unified Health System (SIH/SUS). In addition, population data were obtained through census data and estimates collected on the platform of the Brazilian Institute of Geography and Statistics (IBGE).

The following variables were collected: educational status, sex, race, age group, type of disease, the evolution of the disease, number of hospitalizations, average hospitalization value, the average length of stay, and deaths during hospitalization. Notified cases of neonatal tetanus were excluded. The average amount spent on hospitalization was obtained by the total value of hospitalizations divided by the number of hospitalizations. The average length of stay was obtained by the sum of all days spent divided by the number of hospitalizations.

The lethality rate was calculated using the ratio between the number of cases with evolution to death and the total number of reported cases of tetanus in the same region and year. The tetanus incidence rate was calculated by macro-region and age group.

Statistical analysis used GraphPad Prism version 9.1 (GraphPad Software Inc., San Diego, CA, USA). Descriptive statistics included measures of absolute and relative frequency. Time series were evaluated using parameters estimated through linear regression. The level of significance adopted was 1%.

RESULTS

In Brazil, 2,772 cases of tetanus were reported from 2009–2018 and were distributed as follows: 918 in the Northeast (NE), 625 in the Southeast (SE), 545 in the South (S), 361 in the North (N), and 323 in the Midwest (CO). The three states with the highest numbers of tetanus cases were Minas Gerais, Rio Grande do Sul, and São Paulo.

The reported cases of tetanus in Brazil reduced during 10 years of analysis (325 cases in 2009, declining to 199 cases in 2018). An important finding in the analysis of the time series was the significant reduction in cases in the NE over time (-50%; β= -6.9; p=0.003). The other regions showed stable behavior in the period (Figure 1A).

The overall incidence in Brazil decreased from 1.7 per million inhabitants in 2009 to 0.95 per million inhabitants in 2018. The greatest reduction was in the Midwest region, from 3.3 per million inhabitants in 2009 to 0.75 per million inhabitants in 2018 (Figure 1B). In all these years, the Southeast region had the lowest incidence, and the Northeast (p=0.001) showed a significant decrease.

In the analysis of the time series, there was an increase in lethality by tetanus in Brazil, being 30.77% in 2009 and 40.70% in 2018, with emphasis on the increase of the lethality observed in the Northeast region, with 25.41% lethality in 2009 and 47.54% in 2018 (p=0.007). The highest lethality rate was in the North region at 48.6% in 2016 and the lowest in the Midwest at 4.5% in 2017 (Figure 1C). When the incidence rate was analyzed by age group, the highest rate occurred in those who aged ≥60 years (Figure 1D).

The highest proportion of cases occurred in individuals with low schooling. The male gender predominated in the approximate proportion of six men for each woman (6:1). The most frequent age group was 40–59 years (Table 1).

Due to the high lethality of tetanus, this study further analyzed the epidemiological profile associated with death. The lethality rate was higher among illiterates, whites, females, and those who aged above 65 years (Table 1).

There were 1,993 admissions to the hospital due to tetanus. The average value of each hospitalization was R$ 5,522.61, with an average stay of 17 days and a lethality rate of 19.5% during hospitalization. Table 2 shows distribution according to region.

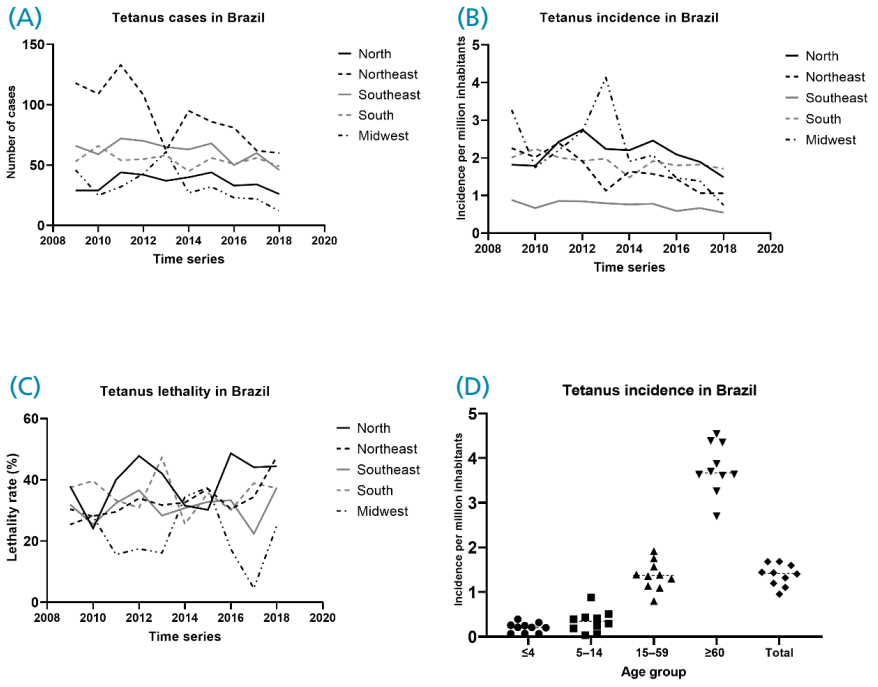

Figure 1. (A) Time series of tetanus cases; (B) tetanus incidence rate per million inhabitants; (C) tetanus lethality rate in Brazil according to macro-region; (D) and tetanus incidence rate in Brazil according to age group.

DISCUSSION

The main findings of the present study show that reported cases of tetanus in Brazil from 2009–2018 predominated in individuals from the 1st–4th grade of elementary school and in males in the approximate proportion of 6:1 when compared with females. The age group most affected was 40–59 years. Our findings are in agreement with other studies carried out in Brazil with other time frames[13].

The higher prevalence in men can be explained by their greater exposure to work activities, in addition to the routine vaccination of pregnant women in prenatal care[14]. The predominance in adults and the elderly people emphasizes that strategies are also needed to update the vaccination calendar of this group, as well as that of the population with low education, and not only in maternal and child vaccinations[15].

The incidence rate for Brazil in 2009 when compared with that of the USA (0.1 per million inhabitants in 2008) was approximately 10 times higher[16]. Some explanations for this finding are that less than 50% of people with acute injuries seek health care in Brazil and some patients fail to receive post-exposure tetanus prophylaxis due to the harmless appearance of some injuries[17]. According to the European Center for Disease Prevention and Control, the incidence rate in Europe in 2017 (0.2 per million inhabitants) was also lower than in Brazil[18].

Despite the still high rate, our study, as well as other studies, showed a reduction from 1.7 per million inhabitants to 0.95 per million inhabitants, from 2009–2018, the largest reduction in the Midwest region. This reduction may be due to greater access to health services, better vaccination coverage, and greater educational and socioeconomic development[14,15].

The highest rate of tetanus incidence in individuals aged ≥60 years is in line with national and international literature[15]. This greater risk is due to inadequate vaccination, as the vaccine is sufficiently immunogenic in the elderly people[16]. Furthermore, over the years, psychomotor capacity and perception of space have decreased, resulting in the elderly people more susceptible to accidents[13].

Great emphasis has been placed on maternal and child vaccination. However, when establishing strategies that focused on certain groups, managers take the risk of neglecting others. Therefore, vaccination campaigns regardless of sex and age, training of health professionals, and observation in each contact as a timely way of immunization are necessary[19]. In addition to vaccination strategies, it is essential to pay attention not only to the risk of tetanus in acute injuries but also to how protocols are formulated for managing chronic injuries that are common in the elderly people[17].

Table 1. Descriptive analysis of tetanus cases and lethality rate reported in Brazil (2009–2018).

	Categories	Cases		Lethality rate
		n	%	%
Educational level (in years)	Illiterate	141	5.09	42.55
	<Grade 4 (elementary school)	418	15.08	33.73
	Grade 4 (elementary school)	160	5.77	31.25
	5–8 (elementary school)	289	10.43	23.53
	Complete elementary school	169	6.10	33.73
	Incomplete high school	83	2.99	25.30
	Complete high school	135	4.87	25.93
	Incomplete graduate level	12	0.43	8.33
	Complete graduate level	26	0.94	30.77
	Not applicable	42	1.52	–
	No data	1,297	46.79	–
Color/race	White	878	31.67	34.97
	Black	225	8.12	32.00
	Yellow	28	1.01	14.29
	Brown	1,417	51.12	31.97
	Indigenous	8	0.29	25.00
	No data	216	7.79	–
Sex	Male	2,360	85.14	31.02
	Female	412	14.86	41.02
Age group (years)	<1	18	0.65	27.78
	01–04	13	0.47	30.77
	05–09	38	1.37	18.42
	10–14	77	2.78	24.68
	15–19	99	3.57	11.11
	20–39	576	20.78	22.92
	40–59	1,106	39.90	31.74
	60–64	255	9.20	34.51
	65–69	220	7.94	45.00
	70–79	277	9.99	47.29
	80 or above	93	3.35	58.06
	1st trimester	1	0.04	0.00
	2nd trimester	2	0.07	100.00
	Without gestational age data	1	0.04	100.00
	No	263	9.49	39.92
	Not applicable	2,486	89.68	31.62
	No data	19	0.69	–
Autochthone	Yes	2,261	81.57	33.83
	No	219	7.90	31.96
	No data	292	10.53	–
Case follow-up	Recovery	1,470	53.03	–
	Death by tetanus	901	32.50	–
	Death by another cause	61	2.20	–
	No data	340	12.27	–

Table 2. Tetanus data from Brazilian Unified Health System (2009–2018).

Macro-region	Hospital admission	Average cost (in R$)	Average stay (in days)
North	313	3,468.32	12.7
Northeast	637	4,688.28	18.1
Southeast	444	7,348.89	19
South	398	6,572.74	16.9
Midwest	141	5,137.1	14.9
Total	1,933	5,522.61	17

The lethality of tetanus in Brazil was 32.5%, similar to Nigeria but much higher than that in developed countries, such as the USA (13.2%)[16] and Japan (6.8%)[20]. The outcome of tetanus cases is primarily related to the quality of care, early diagnosis, and treatment[21,22]. A systematic review of 27 studies involving 3,043 patients showed that the mortality rate in African patients was 43%. This high lethality was because mechanical ventilation is not accessible in many medical facilities[23].

In the present study, the lethality rate was higher in illiterates, whites, females, and those who aged above 65 years old. Evidence has demonstrated a greater chance of progressing to death in female patients (3.46 times more than in men) and in those who aged above 60 years[19].

The average value of each hospitalization was R$ 5,522.61 (Brazilian real BRL), sufficient to purchase about 12,500 vaccines, considering that the value of one dose of the DT vaccine (adult double) was approximately R$ 0.41[24]. This reinforces the cost-benefit of vaccination policies, regardless of age and gender.

Studies have shown the high cost of treatment and support for tetanus. Some developed countries such as the United Kingdom have used the Tetanus Quick Stick (TQS) test, a rapid test to assess serological status in individuals with suspected lesions, to avoid unnecessary expenses with prophylaxis, since less than 50% of patients remember about their vaccination[25]. However, it is necessary to assess the cost-benefit in Brazil.

The wide variation between mortality during hospitalization, length of stay, and costs in Brazilian regions suggest intense variability within the same country in the management of patients with tetanus. Health professionals' knowledge of post-injury prophylaxis and tetanus management is essential. Thus, it is necessary to train and update professionals who work in a hospital environment[24]. These findings could help researchers, health workers, and health system administrators in planning more effective intervention strategies to reduce tetanus incidence across diverse Brazilian regions. In addition, the findings indicate that new policies must be planned considering specific strategies for the most vulnerable population groups.

CONCLUSION

The study findings showed that the tetanus incidence rate in Brazil is declining. However, special attention should be paid to the elderly people, who have the highest incidence rate and the highest lethality. Greater attention is also needed in the northern region, due to the higher lethality of tetanus as well as the predominance of the highest incidence rate in Brazil, in most years.

AUTHORS' CONTRIBUTIONS

LCC: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **CPCM:** Conceptualization, Investigation, Writing – review & editing. **VPR:** Project administration, Data curation, Formal analysis, Validation, Writing – review & editing.

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Translation, cross-cultural adaptation, and validation of the Canadian Diabetes Risk Questionnaire for the Brazilian population

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SUMMARY

OBJECTIVE: The aim of this study was to translate, cross-culturally adapt, and validate the Canadian Diabetes Risk Questionnaire for use in Brazilian Portuguese.

METHODS: This is a Questionnaire validity study conducted at a private university. The Brazilian version of the Canadian Diabetes Risk Questionnaire was developed by means of the processes of translation, back-translation, committee review, and pretesting. Test-retest reliability was measured using the intraclass correlation coefficient and kappa coefficient. Internal consistency was measured using Cronbach's alpha. For construct validity, the total score of the Canadian Diabetes Risk Questionnaire was correlated with the Diabetes Knowledge Scale and the Diabetes Mellitus Risk Questionnaire. Ceiling and floor effects were also evaluated in the present study.

RESULTS: For construct validity and floor and ceiling effect measurements, a total sample of 100 participants was used. For reliability, a subsample of 34 participants out of the total sample was used. We identified adequate values for reliability (kappa between 0.46–1.00 and ICC 0.96) and internal consistency (Cronbach's alpha 0.80). There were significant correlations between the Canadian Diabetes Risk Questionnaire and the Diabetes Mellitus Risk Questionnaire (r_s=0.370, p<0.001), but not the Diabetes Knowledge Scale (r_s= -0.162). No ceiling or floor effects were found.

CONCLUSION: We concluded that in accordance with the best international recommendations, the Brazilian version of the Canadian Diabetes Risk Questionnaire has adequate psychometric properties.

KEYWORDS: Diabetes mellitus. Questionnaire. Primary health care.

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INTRODUCTION

The number of individuals with diabetes mellitus (DM), especially type 2 (DM2), has been increasing in recent years, and it is considered a public health problem in several parts of the world[1]. The identification of individuals at high risk of developing prediabetes and DM2 is important, since it allows interventions focused on reducing the risk of mortality and complications secondary to the disease, which are divided into macrovascular (heart disease, stroke[2], and decreased heart rate variability[3], which implies a worse prognosis) and microvascular (retinopathy, nephropathy, and neuropathy)[4]. Additionally, identifying individuals at high risk of developing DM can promote early glycemic control, which is crucial to prevent the aforementioned complications[2].

In this context, the use of simple implementation and with adequate cost-effectiveness has been encouraged, especially in primary care, to prevent the onset of DM2. Faced with this scenario, the use of questionnaires has been widely explored in the screening of various diseases[5-9].

The Canadian Diabetes Risk Questionnaire (CANRISK), originally created and validated in Canada, was developed by the Public Health Agency of Canada with the aim of identifying the risk of prediabetes or DM2[10]. In Brazil, there is a questionnaire with the objective of also tracking the risk of developing DM2, called the FINDRISC[11]. However, the CANRISK presents more items that may be related to the risk of developing DM2, such as ethnicity and educational level. In complement, the CANRISK has already been cross-culturally adapted and validated for the Chinese population[12]. Thus, considering the importance of instruments that track the risk of developing DM2, especially in the field of primary care, the objective of this study was to translate, cross-culturally adapt, and validate the CANRISK for use in Brazilian Portuguese.

METHODS

Study design

This is a translation, cross-cultural adaptation, and validity study conducted according to the Guidelines for the Process of Cross-cultural Adaptation of Self-Report Measures[13] and the COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN)[14]. Authorization for translation, cross-cultural adaptation, and validation of the CANRISK in Brazilian Portuguese was granted via email by the author of the original version of the questionnaire (Dr. Janusz Kaczorowski).

This research was approved by the institution's Human Research Ethics Committee under opinion number 2.853.570. All participants validated their participation by signing an informed consent form. The recruitment of individuals took place in the university community, in the communities surrounding the university, and in a community association in the city of São Luís (MA, Brazil). The research was disseminated through pamphlets and social media.

Translation and cross-cultural adaptation of the Canadian Diabetes Risk Questionnaire

The process of translating and cross-cultural adaptation of the CANRISK into Brazilian Portuguese followed the criteria of Beaton et al.[13] and was carried out in five phases, as described below:

(1) Translation: two independent translators, both with Brazilian Portuguese as the mother tongue and fluent in English, performed the translation of the original version of the questionnaires into Brazilian Portuguese.

(2) Synthesis of the translations: after discussions and reviews, the two translators, under observation of one of the researchers, synthesized the two versions of the questionnaires translated independently and produced a single version in a consensual way.

(3) Back-translation: two independent translators (without technical knowledge of health issues), both with English as the mother tongue and fluent in Portuguese, performed the translation of the Portuguese version of the questionnaires back into English, without prior knowledge of the original version of the questionnaire.

(4) Analysis of a committee of experts: six diabetes specialists together with the four translators of this study analyzed the original version and the translated, synthesized, and back-translated versions and defined the pre-final version of the CANRISK, which we now call the CANRISK.

(5) Test of the pre-final version: the pre-final version of the questionnaire was applied to 30 individuals without a diagnosis of diabetes and with the Portuguese language as their mother tongue, assessing the understanding of the items and responses of the CANRISK by these respondents.

Participants

To calculate the sample size for this validation study, COSMIN was used, with a minimum number of 100 individuals recommended[14]. As eligibility criteria, we considered individuals of both sexes, without the diagnosis of type 1 or type 2 DM,

aged between 40–74 years, and without cognitive deficits or any other limitations that prevented them from answering the questionnaire.

Canadian Diabetes Risk Questionnaire

The CANRISK is a questionnaire containing 12 items that assess the risk of prediabetes or DM2. It is mainly used for adults aged 45–74 years. The final CANRISK score ranges from 0–87 points and can be categorized as follows: score <21 points, the individual is at low risk; between 21–32 points, the individual is at moderate risk; and >33 points, an individual is at high risk for developing DM2.

Other questionnaires

In addition to the CANRISK, two other questionnaires already adapted and validated for Brazilian Portuguese were applied to carry out construct validity:

(1) Diabetes Knowledge Scale (DKN-A), a questionnaire validated for the Brazilian population by Torres et al.[8], composed of 15 multiple-choice questions on various aspects related to the general knowledge of DM2. The total score was calculated by assigning 1 point for each correct answer, ranging from 0–15. The higher the score, the greater the knowledge about DM2.

(2) Diabetes Mellitus Risk Questionnaire (DMRQ), a questionnaire validated in the master's dissertation by Cruz et al.[15], composed of seven items and with a total score ranging from 0 to 27 points. The higher the score, the higher the risk of developing DM2.

Statistical analysis

Descriptive analysis was initially performed with the presentation of quantitative data using means and standard deviations and qualitative data using absolute numbers and percentages. Comparisons between the total sample and the subsample were performed using the paired t test or χ^2 test.

Test-retest reliability was assessed using the kappa coefficient, intraclass correlation coefficient (ICC), standard error of measurement (SEM), and minimum detectable change (MDC). Internal consistency was assessed using Cronbach's alpha.

The kappa values were interpreted as follows: <0, poor; 0.01–0.20, slight; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, substantial; and 0.81–1, almost perfect[16]. The ICC values were interpreted as follows: for values <0.40, reliability was considered low; between 0.40–0.75, moderate; between 0.75–0.90, substantial; and >0.90, excellent[17]. The SEM percentage was interpreted as follows: ≤5%, very good; >5% and ≤10%, good; >10% and ≤20%, doubtful; and >20%, negative[18].

Kolmogorov-Smirnov normality test was applied; however, due to the non-normal distribution of data, Spearman's correlation coefficient (r_s) was used to determine the magnitude of the correlations between the CANRISK, the DMRQ, and the DKN-A. The magnitude of the correlations was interpreted in accordance with the COSMIN recommendations: correlations with instruments measuring similar constructs should be ≥0.50; correlations with instruments measuring related but dissimilar constructs should be 0.30–0.50; and correlations with instruments measuring unrelated constructs should be <0.30[14].

Ceiling and floor effects were also evaluated in the present study. By definition, these effects occurred when a number of study participants (set as over 15%) reached the minimum or maximum values of the total score of the questionnaire.

All statistical analyses were performed using SPSS software, version 17.0 (Chicago, IL, USA), and a significance level of 5% was adopted.

RESULTS

With regard to translation and cross-cultural adaptation, to facilitate understanding of the questionnaire, the committee of experts suggested the following changes: use the name of the country instead of nationality in the item that addresses the ethnicity of biological parents (item 11) (e.g., change "Chinese" to "China") and inclusion of the country Japan in East Asia in this same item. This adapted version of the CANRISK was then applied to 30 participants to assess the level of understanding of the items. There was 100% understanding of all items in the questionnaire. Thus, we defined the final Brazilian Portuguese version of the CANRISK.

A total of 100 participants was recruited and included in the study. Out of this total sample, a subsample of 34 participants was selected for the test–retest reliability calculations. Table 1 presents the characteristics of the sample, and we observed that most of the participants were women, married, and overweight.

Regarding the reliability (Tables 2 and 3), when considering each item of the CANRISK, we observed adequate values of reliability (kappa ≥0.46). Item 6 was the least reliable (kappa=0.46), and items 1, 2, 3, 4, 5, and 7 were the most reliable (kappa=1.00). Considering the total score, we observed adequate reliability (ICC=0.96) and internal consistency (Cronbach's alpha=0.80).

To assess the construct validity by means of correlation with a validated questionnaire (Table 4), we observed adequate correlations of the CANRISK score with the DMRQ (r_s=0.370). No participants achieved a CANRISK maximum score of 100 or 0. Thus, ceiling and floor effects were not observed.

Table 1. Sociodemographic and clinical characteristics of the participants.

	Reliability phase (n=34)	Validity phase (n=100)	p-value
Age (years)	54.00 (9.50)	53.70 (8.04)	0.130
Gender (female), n (%)	27 (79.4)	79 (79)	1.000
Marital status, n (%)			0.312
Single	11 (32.4)	32 (32)	
Married	20 (58.8)	55 (55)	
Divorced	1 (2.9)	7 (7)	
Widower	2 (5.9)	6 (6)	
Weight (kg)	60.61 (19.01)	65.21 (16.32)	0.696
Height (m)	1.57 (0.09)	1.57 (0.09)	0.999
BMI (kg/m^2)	24.10 (7.44)	26.11 (6.38)	0.173
Schooling, n (%)			
Basic education	13 (38.2)	46 (46)	0.091
High school	15 (44.1)	47 (47)	
Higher education	6 (17.6)	7 (7)	
Physical activity (yes), n (%)	11 (32.4)	33 (33)	1.000
Smoker (yes), n (%)	1 (2.9)	4 (4)	0.787
DKN-A (score)	7.26 (2.76)	7.46 (2.59)	0.889
DMRQ (score)	11.44 (4.22)	11.87 (4.15)	0.360
CANRISK (score)	28.41 (11.36)	31.78 (13.05)	0.081

BMI: body mass index; DKN-A: Diabetes Knowledge Scale; DMRQ: Diabetes Mellitus Risk Questionnaire; CANRISK: Canadian Diabetes Risk Questionnaire. Values are presented in mean (standard deviation) or number (percentage). There was no significant difference between groups (p>0.05, paired *t* test or χ^2 test).

Table 2. Reliability and internal consistency of items of the Canadian Diabetes Risk Questionnaire.

CANRISK item number	Mean (SD)		Kappa	Cronbach's alpha if item excluded
	Test	Retest		
1	8.29 (5.11)	8.29 (5.11)	1.00	0.79
2	1.23 (2.46)	1.23 (2.46)	1.00	0.81
3	3.23 (3.49)	3.23 (3.49)	1.00	0.78
4	3.82 (2.47)	3.82 (2.47)	1.00	0.79
5	0.64 (0.48)	0.64 (0.48)	1.00	0.80
6	0.76 (0.98)	0.94 (1.01)	0.46	0.80
7	1.52 (1.97)	1.52 (1.97)	1.00	0.80
8	2.05 (5.03)	3.29 (6.02)	0.71	0.80
9	0.05 (0.23)	0.02 (0.17)	0.65	0.80
10	1.05 (1.49)	0.94 (1.22)	0.90	0.80
11	3.26 (2.26)	2.79 (1.90)	0.75	0.79
12	2.29 (2.19)	2.26 (2.21)	0.96	0.80

CANRISK: Canadian Diabetes Risk Questionnaire; SD: standard deviation. Values are presented as mean (SD).

Table 3. Reliability of the total score of the Canadian Diabetes Risk Questionnaire.

Test	Retest	ICC (95%CI)	SEM (absolute)	SEM (%)	MDC (absolute)	MDC (%)	Cronbach's alpha
28.41 (11.36)	29.02 (11.41)	0.96 (0.92–0.98)	2.28	7.93	6.31	21.98	0.80

ICC: intraclass correlation coefficient; CI: confidence interval; SEM: standard error of measurement; MDC: minimum detectable change. Data are presented as mean (SD).

Table 4. Correlation between the total score of the Canadian Diabetes Risk Questionnaire and the other questionnaires applied in the study sample (n=100).

Questionnaires	CANRISK
DKN-A	r_s= -0.162, p=0.120
DMRQ	r_s=0.370, p<0.001*

DKN-A: diabetes knowledge scale; DMRQ: diabetes mellitus risk questionnaire. *Statistically significant correlation (p<0.05, Spearman's correlation coefficient).

DISCUSSION

The main findings of this study are that

(i) the CANRISK in the Brazilian Portuguese language has an adequate level of understanding by the population studied and

(ii) the CANRISK presents acceptable values of reliability, internal consistency, and validity, thus confirming the initial hypothesis of the study.

Although the CANRISK is available in 13 other countries (languages: English, French, Chinese, Gujarati, Korean, Persian/Farsi, Punjabi, Spanish, Tagalog, Tamil, Urdu, and Vietnamese)[10], to the best of our knowledge, these translations do not seem to have cross-cultural adaptation and validation in accordance with best international practices[14]. There are only two versions of the CANRISK adapted cross-culturally and validated, one for Chinese with appropriate psychometric properties (CHINARISK)[12] and other for the Arabic version (ARABRISK)[19]. It is also worth mentioning that the ARABRISK performed the test–retest on the same day, which is not an advisable practice according to the COSMIN guideline[14].

Among the psychometric properties that an instrument must have, the COSMIN highlights the

(i) reliability,

(ii) validity (composed of several sub-items, such as face, content, construct, structural, cross-cultural, and criterion validity), and

(iii) responsiveness. Our validation study involved the reliability (using kappa, ICC, SEM, and MDC), cross-cultural validity (using translation, synthesis of translations, back-translation, expert committee, and pre-final version testing), construct validity (using the correlation between questionnaires), and internal structure validity (using Cronbach's alpha)[14]. Thus, such properties already ensure that the CANRISK can be applied to the Brazilian population.

In the Brazilian Portuguese language, the FINDRISC tool is available to tracking the risk of developing DM2[11]. Comparing the results of the Brazilian version of the FINDRISC versus CANRISK, we observed that both have acceptable measurement properties, with ICC values >0.90, kappa values >0.40, Cronbach's alpha values >0.75, and significant correlations (p<0.05) with other instruments already validated in the Brazil. However, the FINDRISC has slightly higher values. Thus, health professionals who are interested in tracking the risk of developing DM2 can choose to use the FINDRISC or CANRISK.

This study has limitations that must be expressed, as well as suggestions. Initially, we recommend testing the cross-cultural adaptation of the CANRISK for other languages based on the COSMIN[14], a fact that greatly limited the discussion of the results. In addition, due to the need for specific methodology, our study did not investigate the accuracy or responsiveness of the CANRISK. Thus, we suggest that future studies measure these properties. We conducted the present study with a specific sample from a city in northeastern Brazil; therefore, we suggest that future studies be carried out testing this Brazilian version of the CANRISK on larger samples and in different regions of the country.

CONCLUSION

The Brazilian Portuguese version of the CANRISK has adequate psychometric properties according to the best scientific recommendations.

AUTHORS' CONTRIBUTIONS

IML: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **ASR:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – review & editing. **JGD:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **MGPB:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **WSBM:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **PRF:** Data curation, Formal Analysis, Methodology, Writing – original draft. **KVBCS:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **LVA:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **RRJT:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **DBD:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – review & editing.

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ORIGINAL ARTICLE
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Effect of group hope therapy on self-efficacy of adolescents with type 1 diabetes

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SUMMARY

OBJECTIVE: This study aims to determine the effect of group hope therapy on the self-efficacy of adolescents with type 1 diabetes.

METHODS: This randomized clinical trial was conducted on 45 adolescent patients with type 1 diabetes in Iran. The subjects were randomly assigned into the intervention and control groups. The intervention group received eight 90-min sessions of group hope therapy using Snyder's method.

RESULTS: The mean score of self-efficacies in the intervention group was significantly higher than the control group ($p<0.05$).

CONCLUSION: The results of the study suggested that group hope therapy had a significant impact on self-efficacy of adolescents with type 1 diabetes but had no effect on academic self-efficacy and self-efficacy in blood glucose management.

KEYWORDS: Hope. Diabetes mellitus. Self efficacy. Iran. Adolescent.

INTRODUCTION

Self-efficacy refers to an individual's belief in his or her capacity to execute behaviors necessary to produce specific performance attainments. Factors that influence diabetes control include the association between self-efficacy and self-care behaviors. When diabetes control is achieved, complications decline and hence quality of life increases[1]. Children and adolescents with type 1 diabetes (T1D) do not have an appropriate emotional response and experience a low-level mental well-being and self-efficacy because of problems such as diet, limited activity, invasive monitoring of blood sugar, daily insulin injections, chronic physical complications, and hospitalization imposed on them by the disease. All these conditions lead adolescents to low quality of life[2]. Hope therapy is a healthcare program designed based on Snyder's theory to increase hopeful thinking and strengthen activities related to goal achievement that is derived from cognitive–behavioral therapies, solution-based treatments, and fictional and narrative treatment[3]. In hope therapy, participants are taught how to set their own goals, create strategies to achieve those goals, develop the motivation for implementing them, and maintain them along the way[4]. Given the fact that group training is effective in increasing patients' motivation to follow treatment recommendations, this treatment is done collectively as group therapy. Although some studies have been conducted to investigate the relationship between self-efficacy, quality of life, and self-care, only few studies have examined the increase of self-efficacy in diabetic adolescents. Accordingly, no study has been found concerning the effect of group hope therapy on self-efficacy in diabetic adolescents. Therefore, the present study was conducted to determine the effect of training group hope therapy on the self-efficacy of the adolescents with T1D.

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METHODS

Sample and study design

The present study is a randomized controlled trial that includes the pre-test, post-test, and 2-month follow-up stages and was conducted from August to November 2019 in Iran. The study population consisted of all adolescents with T1D who had referred to Isfahan Endocrine and Metabolism Research Center (IEMRC). Using convenience sampling method, 46 patients were selected and randomly allocated to the intervention and control groups (23 in each group). Inclusion criteria were T1D, passage of at least 6 months from diagnosis, 13–19 years old, no participation in other psychotherapy programs, no other physical illness, no mental illness or crisis, and no severe mental stress including death of a parent or sibling, parental divorce, and severe accident in the past 6 months. The samples were selected from files of the patients with T1D in IEMRC. Participants and their parents signed a consent form. The members of the intervention group were randomly divided into two groups, each consisting of 11 and 12 to participate in hope therapy sessions, which were held in eight 90-min sessions twice a week. The contents of the classes were based on 2000 Snyder's hope therapy program (Table 1). No intervention was performed for the control group, and they received only routine diabetes self-care training provided by the center itself.

Self-efficacy questionnaires were completed three times, that is, before the intervention, immediately after completing the eight treatment sessions of the intervention group, and 2 months later by all participants of the study.

Measures

The demographic characteristics questionnaire and the Self-Efficacy Questionnaire for Children (SEQ_C), which has been developed by Muris in 2001 based on the Bandura Self-Efficacy Questionnaire, consist of 23 questions in three categories: social, educational, and emotional self-efficacy. The Cronbach's alpha coefficient is 0.80 and the reliability is 0.70[5]. The Diabetes Management Self-Efficacy Scale (DMSES) was developed in 1999 by Van der Bijl et al. and consists of 19 questions in four categories. The Cronbach's alpha coefficient for this instrument is 0.92 and its reliability is 0.89[6].

Statistical analysis

Independent *t*-tests (quantitative variables), Mann-Whitney U test (ranked qualitative variables), and chi-square or Fisher's exact test (nominal qualitative variables) were used to compare the demographic characteristics between the two groups.

Ethical considerations

The research ethics committee of Isfahan University of Medical Sciences (IR.MUI.RESEARCH.REC.1398.365) approved the study. This study was registered at the Iranian Registry of Clinical Trials (IRCT20190813044520N1).

RESULTS

The mean age of the participants was 16.36±0.45 years and the duration of illness was 6.38±0.86 years. Regarding demographic variables, such as age, gender, diabetes history, educational level, and parental characteristics, there was no significant difference between the two groups ($p>0.05$). The self-efficacy of the intervention group was higher than that of the control group after the intervention, and according to the experiment conducted in the next two months, an increase in self-efficacy was persistent in the intervention group (Table 2). Repeated-measures analysis of variance (ANOVA) showed that group hope therapy has no effect on the component of educational self-efficacy but has increased self-efficacy in the social and emotional domains of the adolescents (Table 3). Also, there has been no effect on the field of blood sugar measurement but has increased self-efficacy in the domains of diet, physical activity, and drug administration of the adolescents (Table 4).

Table 1. General outline of hope therapy program for diabetic patients.

Sessions	Content
1	Initial familiarization with the participants
2	The importance of having hope in life, its role in the problem-solving process, familiarizing the subjects with the features and effects of hope in life
3	Listening to the life story of each member of the group
4	Expressing the strengths of each member from the perspective of other group members
5	Introducing and writing a list of current events and important aspects of life by each member
6	Expressing the characteristics of goal setting based on Snyder's theory in optimistic subjects
7	Choosing the right solutions by each person to achieve the preset goals and teaching how to turn the pathways into small steps
8	An overview of the conducted interventions and providing solutions to create and maintain motivation

Table 2. Comparison of the mean total score of children and adolescents' self-efficacy and diabetes management self-efficacy between the two groups at three times.

Component	Group	N°	Before the intervention		Immediately after the intervention		Two months after the intervention		Repeated-measures ANOVA		
			M	SD	M	SD	M	SD	F	df	p
Child and teenager self-efficacy	Intervention	23	80.96	10.37	86.52	11.63	89.95	13.17	7.34	20, 2	0.004
	Control	22	81.41	11.10	80.14	10.75	79.38	12.55	0.75	19, 2	0.480
Diabetes management self-efficacy	Intervention	23	131.25	31.32	153.26	35.01	154.73	36.79	8.76	20, 2	0.002
	Control	22	129.86	34.29	129.91	34.49	128.81	37.67	0.02	19, 2	0.980

ANOVA: analysis of variance; df: degrees of freedom; SD: standard deviation.

Table 3. Comparison of the mean total score of children and adolescents' self-efficacy and its domains between the two groups at three times.

Domains of child and teenager self-efficacy	Group	Before the intervention		Immediately after the intervention		Two months after the intervention		Repeated-measures ANOVA		
		M	SD	M	SD	M	SD	F	df	p
Total score	Intervention	80.96	10.37	86.52	11.63	89.95	13.17	7.34	20, 2	0.004
	Control	81.41	11.10	80.14	10.75	79.38	12.55	0.75	19, 2	0.480
Social	Intervention	28.96	4.32	33.22	4.45	34.59	4.95	29.81	20, 2	<0.001
	Control	29.73	4.93	29.54	4.90	29.29	4.99	0.14	19, 2	0.870
Educational	Intervention	30.43	4.76	30.09	4.89	30.41	5.69	0.25	20, 2	0.780
	Control	31.32	4.72	30.82	4.33	30.29	4.73	0.91	19, 2	0.420
Emotional	Intervention	21.57	4.74	23.22	4.45	24.95	5.08	5.18	20, 2	0.010
	Control	20.36	5.21	19.77	4.92	19.81	5.41	0.54	19, 2	0.590

ANOVA: analysis of variance; df: degrees of freedom; SD: standard deviation.

Table 4. Comparison of the mean total score of diabetes management self-efficacy and its domains between the two groups at three times.

Domains of child and teenager self-efficacy	Group	Before the intervention		Immediately after the intervention		Two months after the intervention		Repeated--measures ANOVA		
		M	SD	M	SD	M	SD	F	df	P
Total score	Intervention	131.25	31.32	153.26	35.01	154.73	36.79	8.76	20, 2	0.002
	Control	129.86	34.29	129.91	34.49	128.81	37.67	0.02	19, 2	0.980
Diet	Intervention	44.96	17.31	57.52	19.01	58.86	21.75	9.52	20, 2	0.001
	Control	44.82	19.47	45	19.57	45.48	19.69	0.17	19, 2	0.840
Glucometry	Intervention	31.91	7.29	32.74	8.91	32.50	8.42	0.22	20, 2	0.800
	Control	31.32	6.07	30.86	6.62	31	7.25	0.21	19, 2	0.810
Physical activity	Intervention	30.38	6.76	34.52	7.34	34.14	7.29	6.08	20, 2	0.009
	Control	29.64	9.58	29.59	8.35	28.67	8.52	0.16	19, 2	0.850
Drug administration	Intervention	24	4.48	28.48	4.57	29.26	2.22	20.79	20, 2	<0.001
	Control	24.09	6.29	24.45	6.41	23.67	7.95	0.49	19, 2	0.620

ANOVA: analysis of variance; df: degrees of freedom; SD: standard deviation.

DISCUSSION

This study investigated the effect of group hope therapy on self-efficacy of the adolescents with T1D. The results showed that group hope therapy based on Snyder's theory has a significant effect on self-efficacy of the adolescents with T1D. This finding was consistent with the results of previous studies[7-11]. For example, the study conducted by Karimi et al. revealed that hope therapy had 28% increase in self-efficacy of patients with diabetes[12]. During hope therapy and after setting a goal, people look for ways to achieve the goal by assessing obstacles and barriers and thinking about ways to overcome them. This helps individuals find their abilities and motivations, in which indirectly makes them confident and increases their self-efficacy[11]. However, the results of the present study are not in line with the study conducted by Moayed et al. Although hope therapy training in their study increased self-efficacy, its effect was not significant[13]. This difference can be attributed to factors such as the effect of personality traits of each sample on the results, gender of the samples, and different follow-up methods.

Another finding of the present study was that despite the increase in self-efficacy of measuring blood sugar in the intervention group, the effect of hope therapy was not significant, which is consistent with the results obtained by Datye et al.[14] They found that psychological interventions had no or a moderate effect on self-care behaviors and blood glucose measurements in adolescents with diabetes. Furthermore, Dehghan et al. showed that there was no relationship between blood sugar control and diabetes self-efficacy and the effective factor in controlling blood sugar was the duration of diabetes[15]. Similar results were obtained in the study by Beckerle et al.[1] They showed that self-efficacy and self-care were not significantly correlated with blood sugar control and this result could be related to patients' treatment and medication regimens. Although patients measured their blood sugar levels and considered their ability to be at an acceptable level, if their medication levels were not appropriate, their blood sugar levels would not be properly controlled[1]. This was one of the limitations of the present study since the drug regimen has not been studied.

In contrast, the results of the present study are not consistent with the study by Van Allen et al., showing that changes in hope therapy affect both hemoglobin A1C levels and self-control of blood sugar positively[16]. In addition, Santos et al. have demonstrated that the high levels of hope are directly related to blood sugar control in the adolescents with diabetes[17]. This difference in results can be attributed to factors such as different measurement tools, as the present study was an interventional study investigating the self-efficacy of the adolescents in measuring blood sugar, whereas the abovementioned studies were descriptive studies evaluating the level of hemoglobin A1C as the means of controlling blood sugar.

With regard to educational self-efficacy, it was found that hope therapy did not have a significant effect on this variable that is not consistent with previous studies. Tian et al.[11] and Bayanfar et al.[10] have shown that group hope therapy has a positive effect on educational self-efficacy. Feldman and Kubota also showed that hope has a positive relationship with educational achievement[18]. In explaining this finding, in addition to the differences in the age of the samples in the present study and the studies mentioned, it should be noted that in the present study, self-efficacy was examined in different areas and did not focus solely on educational self-efficacy. Moreover, the present study explored educational self-efficacy in the adolescents with diabetes, while the samples in the mentioned studies did not have any illness. Of course, differences in the number of samples and different cultures of countries can also cause this discrepancy.

Although this study tried to control confounding variables by random allocation of the participants to the intervention and control groups, individual differences in mood, nutrition, and family support may possibly have influenced the results of this study in some way. Receiving information from other sources such as the Internet and television or from the intervention group, familial stress, as well as the level of interest and cooperation of the samples, and the optimal and correct application of the learned methods were among the uncontrollable intervening variables in this study.

CONCLUSIONS

The results indicate that group hope therapy could be used as an effective method in increasing the self-efficacy of the adolescents with T1D. However, in the field of educational self-efficacy and self-efficacy in measuring blood sugar, it has not been effective. This inefficacy can be attributed to ways of adapting to the disease, changes in puberty, and also changes in the influence of parents and peers on the decision-making of adolescents.

AUTHORS' CONTRIBUTIONS

NNE: Conceptualization, Data curation, Formal Analysis, Resources, Writing – original draft, Writing – review & editing. **ST:** Conceptualization, Data curation, Formal Analysis, Resources, Writing – original draft, Writing – review & editing. **SJM:** Conceptualization, Data curation, Formal Analysis, Resources, Writing – original draft, Writing – review & editing. **NM:** Conceptualization, Data curation, Formal Analysis, Resources, Writing – original draft, Writing – review & editing.

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Hepatitis C virus eradication on glycemic control and insulin resistance

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SUMMARY

OBJECTIVE: To review data regarding the effects of hepatitis C virus eradication on glycemic control and insulin resistance.

METHODS: This is an integrative literature review, carried out in the PubMed, SciELO, and Lilacs databases. Studies published in the past five years that were fully available, written in English or Portuguese, and have addressed the effects of eradication of the hepatitis C virus on glycemic control and insulin resistance were selected.

RESULTS: Nine studies were selected. Among the results found, it was observed that there is no consensus on the effects of viral eradication on glycemic control and IR, as some authors show an eventual improvement in insulin resistance and glycemic control, while other studies indicate that there are no significant differences between the parameters evaluated after viral eradication.

CONCLUSIONS: Although there is a relationship between hepatitis C virus infection and the development of insulin resistance and type 2 diabetes mellitus and recent advances in research, it was observed that there is no consensus on improving insulin resistance and glycemic control after antiviral treatment, probably due to methodological differences between studies. However, it emphasizes the need to guide people diagnosed with hepatitis C, regarding changes in lifestyle, encouragement of multidisciplinary monitoring, and control of other risk factors.

KEYWORDS: Hepatitis C. Insulin resistance. Diabetes mellitus.

INTRODUCTION

According to the Guidelines of the Brazilian Society of Diabetes[1], there is an increased risk of type 2 diabetes mellitus (T2DM) and insulin resistance (IR) in patients with chronic viral hepatitis, with T2DM being considered one of the most common causes of extrahepatic disease in hepatitis C virus (HCV) infection.

Serfaty[2] reported that the prevalence of T2DM can range from 13.6–67.4% in cases of HCV infection, values higher than those observed for individuals with other chronic liver diseases. Husseim et al.[3] observed a high prevalence of T2DM associated with HCV infection and an increased risk of developing T2DM in individuals with risk factors for metabolic syndrome in the presence of viral infection.

Shawky et al.[4] recruited 60 non-diabetic Egyptian patients with chronic HCV infection to assess IR. Participants were divided into three groups as follows: Group 1 included 30 patients with chronic HCV infection without cirrhosis, Group 2 included 30 patients with chronic HCV infection and liver cirrhosis, and Group 3 included 30 control volunteers. Fasting glucose and insulin levels were found to be significantly higher in Group 2 than those in Groups 1 and 3.

HCV is able to promote IR through several pathogenic pathways, including direct inhibition of intracellular insulin signaling, oxidative stress, activation of inflammatory pathways, modulation of incretins, and dysfunction of pancreatic β cells. The virus can impair the insulin signaling

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pathway through several mechanisms, among which are a stimulus for the production of tumor necrosis factor-alpha (TNF-α), in which high levels in patients with HCV are directly associated with the levels of HOMA-IR and the degree of steatosis, and the serine phosphorylation of insulin receptors[1,5].

T2DM is related to the development of implications and worsens the situation of individuals with hepatitis C in all stages of the disease, even after liver transplantation. The diverse effects related to T2DM are decreased response to antiviral treatment, rapid progression from fibrosis to cirrhosis, and greater chances of developing hepatocellular carcinoma, which may lead to a decrease in the survival rate[6,7].

Although it has already been shown that direct antiviral agents (DAAs) help to eradicate HCV in most patients, there is not much information about viral eradication in hepatic and extrahepatic damage[8]. Therefore, knowing the relationship between HCV infection and the development of IR and T2DM, and due to the controversies of studies in individuals who underwent antiviral treatment, the purpose of this integrative review was to collect data on the effects of HCV eradication on glycemic control and in IR.

METHODS

This is an integrative review, carried out from April to June 2021.

The guiding question was elaborated using the PICO strategy, as proposed by Santos et al.[9], considering P – patients with hepatitis C; I – use of antivirals; C – not applicable to this study; and O – improvement in glycemic control and IR after viral eradication. Thus, the design of this study arose from the following question: in patients with hepatitis C, does viral eradication improve glycemic control and IR?

PubMed, SciELO, and Lilacs databases were used with the following indexed descriptors and their respective synonymies of Medical Subject Headings (MeSH), as well as the following connectives:

1. Hepatitis and;
2. Diabetes mellitus and;
3. Insulin resistance.

For selection, initially, there was the identification stage, with the reading of the publication titles; then, in the screening stage, the abstracts were read to exclude studies that did not meet the research purpose and duplicate publications; at the eligibility stage, the articles were read in full to select those that would be included in this review.

As inclusion criteria, studies published in the past five years that were fully available, written in English or Portuguese, and have addressed the effects of eradication of the HCV on glycemic control and IR were selected. Regarding exclusion criteria, literature review studies, case reports, letters, and editorials were excluded.

RESULTS

Figure 1 shows the stages for selecting the articles. In total, 65 studies were found, of which nine were included in this review (Table 1).

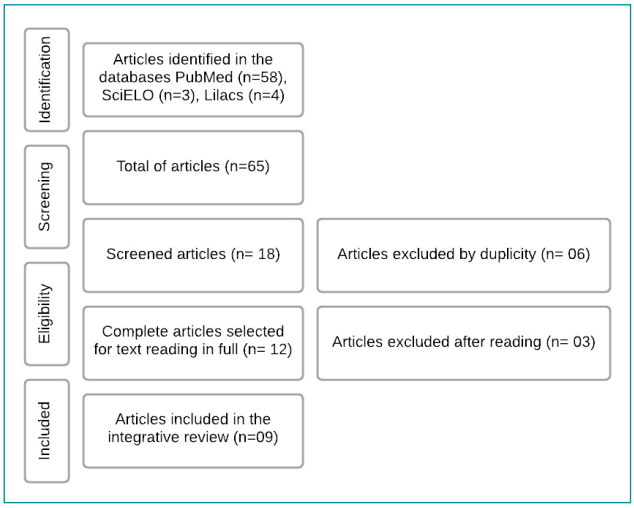

Figure 1. Eligibility flowchart.

Table 1. Study type and sample size of the selected articles.

Author; Country	Study type	Sample size
Andrade et al.[10]; Brazil	Prospective	150 patients
Ciancio et al.[12]; Italy	Case–control	122 patients
Gastaldi et al.[14]; Switzerland	Intervention	17 patients
Gualerzi et al.[13]; Italy	Prospective cohort	82 patients
Lanini et al.[8]; London	Historical cohort	205 patients
Laurito et al.[11]; Brazil	Multicentric, retrospective	200 patients
Li et al.[15]; the United States	Cohort	5,127 patients
Stine et al.[17]; the United States	Retrospective cohort	175 patients
Strauhs-Nitsch et al.[16]; Brazil	Prospective	75 patients

DISCUSSION

Viral eradication effects on glycemic control and insulin resistance

Lanini et al.[8] conducted a cohort study in patients with HCV-related liver diseases and provided evidence that DAA therapy can have positive effects on glycemic control in patients with chronic hepatitis C even when liver cirrhosis is already established. In addition, metabolic improvement may still persist after the end of therapy. However, despite the improvements, blood glucose remained higher in patients with diabetes compared to those without diabetes, indicating that HCV is not the only factor involved in the pathogenesis of diabetes.

Andrade et al.[10] aimed to evaluate the HOMA-IR in patients with hepatitis C treated with DAAs in the sustained virological response (SVR) and observed an increase in mean glucose in the general study population (baseline: 100.65±19.7 *versus* after treatment: 102.36±24.52) and in the HOMA-IR (baseline: 3.69±2.99 *versus* after treatment: 3.72±3.26), but there was a decrease in insulin levels (baseline: 14.46±10.26 *versus* after treatment: 14.03±10.32). Regarding the Delta HOMA-IR, a negative value was observed in the evaluation after drug treatment, but there was no significant difference. When excluding diabetic patients and those with normal HOMA-IR values (<2.5), the mean values of glucose, insulin, HOMA-IR, and Delta HOMA decreased after treatment, the latter with a significant reduction (p=0.02).

Laurito et al.[11] evaluated the impact of IR on SVR in 200 patients with HCV genotype 3 treated with peg-interferon plus ribavirin. Lower IR values were observed in individuals with SVR when compared to those without SVR (2.82±2 *versus* 3.54±2.1; p=0.004). When the univariate analysis was performed, patients with HOMA-IR≥2.5 were 2.6 times less likely to achieve SVR (OR 2.63, 95%CI 1.336–5.175, p=0.005). However, in the multivariate analysis, the HOMA-IR did not reach significance as a predictor of SVR, observing an association only with age and tendency with advanced fibrosis.

Ciancio et al.[12] found that SVR induced significant improvements in fasting glucose and glycated hemoglobin rates in diabetic patients with HCV, thus aiding in glycemic control, despite the observed weight gain.

Gualerzi et al.[13] observed an improvement in the parameters assessed for glucose metabolism in individuals with HCV soon after antiviral treatment. A significant decrease in glucose and insulin levels was found, leading to a reduction in HOMA-IR values (pretreatment: 3.42 [2.66–5.38]; post-treatment: 2.80 [1.78–3.95]; p<0.001), increase in insulin sensitivity (pre: 0.49 [0.26–0.75]; post: 0.64 [0.42–0.91]; p<0.001), and a significant reduction in insulin secretion (pre: 1363 [959–1730]; post: 1264 [976–1588]; p=0.027).

Evaluating interferon-free antiviral therapy (IFNα) in improving liver and peripheral insulin sensitivity associated with HCV, Gastaldi et al.[14] found that complete suppression of virus replication induced by an IFNα-free regimen in lean patients with chronic hepatitis C without fibrosis improves extrahepatic (but not hepatic) insulin sensitivity.

Li et al.[15] evaluated the impact of HCV treatment response in the presence of cirrhosis and other factors on the incidence of T2DM. The authors found that SVR reduced the risk of T2DM by 21%. Therefore, the incidence of diabetes was significantly lower among patients who achieved SVR than those who failed treatment, demonstrating that successful treatment significantly reduces the incidence of T2DM. In addition, patients with a body mass index (BMI) ≥30 kg/m² had an almost four times higher risk of T2DM than patients with a BMI<25 kg/m², and individuals with cirrhosis had a 1.5 times higher risk of T2DM than those without cirrhosis. Also, there is a higher risk for T2DM in patients of African-American, Asian, Native American, or Pacific Islander origin.

In contrast, when comparing the HOMA-IR results of 75 patients with chronic hepatitis C before and after treatment with DAA, Strauhs-Nitsh et al.[16] observed a total of 41.3% of patients with IR before treatment and 52% after the end of medication, with no statistically significant difference (p=0.077). When diabetic patients were excluded, there was also no statistical change in the HOMA-IR results before and after the SVR (p=0.497), that is, there was no improvement in the IR according to the HOMA-IR after obtaining the SVR.

Stine et al.[17] investigated whether treatment with DAA leads to improved post-treatment IR and observed that despite chronic HCV being associated with increased IR, there was no significant difference (p=0.268) between the level of glycated hemoglobin (HbA1c) in the pretreatment (7.36 mg/dL, 95%CI 6.55–8.16) and post-treatment (7.11 mg/dL, 95%CI 6.34–7.88) of diabetic individuals with and without cirrhosis. In addition, 23.1% were taking insulin therapy in pretreatment, compared to 42.3% in post-treatment, and 53.8% were taking oral T2DM medications before treatment, compared with 57.7% after completion, but there was no statistically significant difference. Thus, the authors emphasized that HbA1c was not affected by viral eradication; however, this result can be attributed to the clinical management of diabetes and hyperglycemia with pharmacological therapy, since increasing doses of antihyperglycemic drug use were observed, suggesting the need for long-term studies to comprehend the relationship between viral eradication and insulin metabolism.

This study has limitations. First, methodological differences for the assessment of IR and glycemic changes made it difficult to compare the results. Second, there was lack of a cutoff point to classify the presence of IR by the HOMA-IR. Furthermore, the

samples varied in terms of fibrosis staging, which also interfered with the results observed. Thus, new intervention studies are suggested to identify whether there is improvement in IR and glycemic control in patients after the eradication of the HCV, emphasizing the need to establish a methodological standard in future research.

CONCLUSIONS

Although there is a relationship between HCV infection and the development of IR and T2DM and recent advances in research, it was observed that there is no consensus on the improvement of IR and glycemic control after antiviral treatment, possibly due to methodological differences between the studies. However, it emphasizes the need to guide people diagnosed with hepatitis C regarding lifestyle changes, in addition to encouraging multidisciplinary monitoring with periodic biochemical tests and control of other risk factors that may interfere with glycemic control.

AUTHORS' CONTRIBUTIONS

MMLC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **JLLD:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **DLG:** Conceptualization, Methodology, Supervision, Writing – review & editing. **JASQ:** Conceptualization, Methodology, Supervision, Writing – review & editing.

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Side effects and antibody response of an inactive severe acute respiratory syndrome coronavirus 2 vaccine among health care workers

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SUMMARY

OBJECTIVE: This study aims to investigate the antibody response and the side effects of the two-dose inactive SARS-CoV-2 vaccine (CoronaVac, Sinovac, China) among a health care worker population in Turkey.

METHODS: This study was a prospective, cross-sectional, single-center study conducted between December 16, 2020, and March 15, 2021. We evaluated the side effects from a questionnaire, and anti-spike immunoglobulin G response to the vaccine (0- and 28-day schedule) using an enzyme-linked immunosorbent assay.

RESULTS: A total of 94 of 184 health care workers completed this study. The percentages of participants who were seronegative at baseline and achieved to the seropositivity were 21.3 and 97.9%, respectively, on day 21 after vaccinations. The seropositivity was predominantly detected in 31–45 years of the age group (55.4%, p=0.636), normal body mass index (47.8%, p=0.999), nonsmokers (64.1%, p=0.999), those without any comorbidities (73.9%, p=0.463), and those without any side effects (70.2%, p=0.256). The frequencies of overall side effects within seven days after the first and second doses of CoronaVac were 37.2 and 28.7%, respectively. The most common side effects was localized pain at the injection site (15.7 and 11.6%, respectively).

CONCLUSIONS: We found that vaccination by two-dose CoronaVac could elicit a specific humoral response, and it was well tolerated in health care workers. The high seropositivity developed after the second dose attracted attention. Our study will be useful in terms of showing short-term immunity and side effects.

KEYWORDS: COVID-19. Pandemics. Vaccine. Adverse events. Health personnel.

INTRODUCTION

Since the development of the first vaccine for smallpox 225 years ago, vaccinations have been saving three million people every year. However, radical changes in population density, nutrition, travel habits around the world, climate change, and ecosystem degradation are leading to the emergence of old and new pathogens that pose a risk of pandemic threats. The current pandemic, the coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to 198.2 million laboratory-confirmed cases, with more than 4.2 million deaths[1,2]. As of August 2, 2021, in Turkey, 5.75 million people have been infected with

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SARS-CoV-2, and 51.43 of them have died[2]. At the same time period, in Brazil, 19.918 million people have been infected, and 556.37 of them have died[2]. There are currently more than 200 preclinical and clinical vaccine candidates with various antigen delivery systems such as non-replicating viral vector, protein subunit, mRNA, and inactivated virus[3]. However, a limited number of them (currently, six), which has their own advantages and disadvantages, had emergency use authorization (EUA) including CoronaVac[4]. A CoronaVac study among fully immunized people (≥14 days after receipt of the second dose) in Chile reported that the effectiveness of the vaccine was 65.9% for the prevention of COVID-19 and 87.5% for the prevention of hospitalization, 90.3% for the prevention of intensive care unit admission, and 86.3% for the prevention of COVID-19-related death. This study is invaluable as it reflects real-life data[5]. Few studies have examined antibody response for the vaccine efficacy among health care workers (HCWs)[6-9].

In this study, we investigated the anti-spike antibody response to the two-dose CoronaVac and the side effects (SEs) experienced within 7 days of each postvaccination period among an HCW population in Turkey.

METHODS

Study design and participants

This is a prospective cross-sectional study conducted between December 16, 2020, and March 15, 2021, at a 576-bed tertiary university hospital using 184 HCWs who had a negative test result before vaccinations. Of 184 HCWs, 94 who did not meet any of the exclusion criteria completed the study. Blood samples of eligible participants were collected after 20 days following each dose of vaccinations. Signed informed voluntary consent was obtained from all participants. We recorded demographic characteristics, medical history, smoking habit, whether they experienced any SEs, and needed treatment during the seven days after each dose of the vaccination by a questionnaire.

Exclusion criteria were as follows:

- COVID-19 polymerase chain reaction (PCR) test positivity or anti-SARS-CoV-2 immunoglobulin G (IgG) test result as borderline/positive at any time during the pandemic,
- Compatible COVID-19 symptoms (i.e., fever, cough, and dyspnea) in the past three months,
- Any compatible symptoms or PCR positivity after the first dose of CoronaVac,
- Changed the decision to have a second dose for whatever reason.

The classification of obesity according to body mass index (BMI) was made by the World Health Organization (WHO) classification[10].

The grading (severity) scales of SEs are Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), and Grade 4 (life-threatening)[11].

Serological test

The antibody response was tested using an enzyme-linked immunosorbent assay (Euroimmun, Medizinische Labordiagnostika, Germany), which provides semi-quantitative determination of anti-SARS-CoV-2 IgG against the S1 domain of the spike protein. Serum samples with a result of ≥1.1 were considered positive, a result of ≥0.8–<1.1 as borderline, and a result of <0.8 as negative in accordance with the manufacturer's instructions. Borderline results were considered negative for analysis.

Statistical analysis

Descriptive statistics for the categorical variables were given as frequency (n) and percentage (%). Categorical variables were compared using Fisher–Freeman–Halton test or Pearson's χ^2 test. Statistical analysis was performed using Statistical Package for the Social Sciences version 25.0 package program (SPSS, IBM, USA). A $p<0.05$ was set as statistically significant.

RESULTS

Demographic characteristics and comorbidities of HCWs

A total of 94 participants of 184 HCWs with a predominance of male (54.3%) met the necessary criteria for the study. All the participants were Turkish nationals. The age of the study group ranged from 22–54 years, with a mean of 41.03±7.74 (22–54). The demographic characteristics, comorbidities, and smoking status of participants are demonstrated in Table 1.

Antibody response

The proportion of participants who were seronegative at baseline and achieved antibody positivity on day 21 after the first dose of vaccination was 21.3%. The seropositivity rate increased from 21.3–97.9% after the second dose of vaccine. The majority of seropositivities were found in the 31–45 years of the age group after both doses of vaccine (70%, p=0.166; 55.4%, p=0.636, respectively). In a comparison of the seropositivity rates between genders, the predominance of people were women (65%) after the first dose (p=0.158) and men (53.3%) after the second dose (p=0.498). The majority of seropositivities were found in those who had normal BMI (18.5–24.9) after both doses of vaccine (50–47.8%, p=0.932; p=0.999, respectively).

The seropositivities were found predominantly in those who were not smokers after each dose of vaccine (75–64.1%, p=0.555 and p=0.999, respectively). The rates of seropositivity in HCWs without any comorbidities were higher after each of two doses (60%, p=0.142; 73.9%, p=0.463, respectively). Among the participants who did not develop any antibody response after the first dose, 9.5% had an endocrine disease (five HCWs with hypothyroidism and two HCWs with diabetes mellitus) and 8.1% had a cardiovascular disease (hypertension) (p=0.007). However, these participants developed a higher rate of antibody response after the second dose (7.5–9.4%, respectively) (p=0.015) (Table 2).

Table 1. Demographic characteristics and comorbidities of the health care workers.

Items	Results (n=94)
Age (years), mean values±SD (min–max)	41.0±7.74 (22–54)
Sex (F/M), n (%)	43/51 (45.7/54.3)
Weight (kg), mean values±SD	72.86±12.74
Height (cm), mean values (min–max)	170 (155–190)
BMI (kg/m^2), mean values±SD	24.95±3.25
BMI groups, n (%)	
18.5–24.9 (Normal)	45 (47.9)
25–29.9 (Preobesity)	42 (44.7)
30–34.9 (Obesity)	7 (7.4)
Age groups, n (%)	
18–30	11 (11.7)
31–45	53 (56.4)
45–60	30 (31.9)
Comorbidities	
None	69 (68.3)
Cardiovascular diseases	11 (10.9)
Rheumatic diseases	2 (2.0)
Endocrine diseases	9 (8.9)
Autoimmune diseases	3 (3.0)
Malignancy	3 (3.0)
Other diseases*	4 (4.0)
Smoking status, n (%)	
Non-smoker	60 (63.8)
Smoker	34 (36.2)

SD: standard deviation; F/M: female/male; BMI: body mass index. *Other diseases include uveitis, seborrheic dermatitis, endometriosis, asthma, and migraine.

Side effects

The frequencies of overall SEs within the first seven days after vaccinations were 37.2% (35/94) and 28.7% (27/94), respectively. The most common SE was localized pain at the injection site, which accounted for 15.7% and 11.6%, respectively. Headache (10.7%) was the most common systemic SE following localized pain. The distribution of multiple types of SEs after vaccinations is shown in Table 3. Nearly all the SEs were mild or moderate (Grade 1/2) in intensity. After the first dose, 5.3% (n=5) of the participants used oral acetaminophen tablets for headache. After the second dose, 7.5% (n=7) of the participants required oral therapy (six participants needed oral paracetamol for headache, one sublingual captopril for hypertension). Sixty percent of those who received treatment due to the SEs after the first dose of vaccine also required treatment after the second dose. The antibody positivity rates were higher (70.2%, p=0.256) in participants without any SEs after a second dose of CoronaVac, and their seroconversion rate was also slightly higher (66.3%, p=0.222). However, in the analysis of dependency status between antibody responses and variables (age groups, sex, BMI, smoking habits, comorbidities, and presence of SEs), a statistically significant relationship was found along with comorbidities after both doses of vaccination (p=0.007 and p=0.015, respectively) (Table 2).

DISCUSSION

Our study showed that the vaccination by two-dose CoronaVac administered 28 days apart elicits a specific humoral response, and it was well tolerated as all SEs experienced were mild and moderate in severity in agreement with the clinical phase trials[12-17].

We found that seropositivity after the first dose of vaccination (21.3%) was low, but it achieved a higher percentage of 97.9% after the second dose. This rate was slightly higher than the clinical phase trials[13,14]. In a recent study in Turkey, the seropositivity after the first dose was reported higher (77.8%) than ours, but it concurs with our finding after the second dose (99.6%). The higher rate of seropositivity after the first dose was because those who had before COVID-19 were included in their study and there was a methodological difference[6]. The high seropositivity rate we found (97.9%) is important and a promising finding; however, today, we know that in the case of some mutations in the S protein, which causes new virus variants (P.1, P.2, B.1.351, B.1.1.7, B.1.325, B1.617, etc.), the variants not only change transmissibility and clinical severity of disease but also affect the susceptibility of the virus to naturally or vaccine-induced immunity (especially E484K and

Table 2. Antibody response after each doses of CoronaVac according to the variables.

	After the first dose of vaccine				After the second dose of vaccine				Ratio of antibody index value increase		
	Negative	Borderline	Positive	p-value	Negative	Borderline	Positive	p-value	<4-fold	≥4-fold	p-value
Age groups (years), n (%)											
18–30	6 (9.0)	2 (28.6)	3 (15.0)	0.166[a]	–	–	11 (12.0)	0.636[a]	2 (11.8)	9 (11.7)	0.356[b]
31–45	36 (53.7)	3 (42.9)	14 (70.0)		–	2 (100.0)	51 (55.4)		12 (70.6)	41 (53.2)	
45–60	25 (37.3)	2 (28.6)	3 (15.0)		–	–	30 (32.6)		3 (17.6)	27 (35.1)	
Sex, n (%)											
Female	27 (40.3)	3 (42.9)	13 (65.0)	0.158[a]	–	–	43 (46.7)	0.498[a]	11 (64.7)	32 (41.6)	0.083[b]
Male	40 (59.7)	4 (57.1)	7 (35.0)		–	2 (100.0)	49 (53.3)		6 (35.3)	45 (58.4)	
BMI groups (kg/m^2), n (%)											
18.5–24.9	31 (46.3)	4 (57.1)	10 (50.0)	0.932[a]	–	1 (50.0)	44 (47.8)	0.999[a]	9 (52.9)	36 (46.8)	0.128[b]
25–29.9	30 (44.8)	3 (42.9)	9 (45.0)		–	1 (50.0)	41 (44.6)		5 (29.4)	37 (48.0)	
30–34.9	6 (8.9)	–	1 (5.0)		–	–	7 (7.6)		3 (17.6)	4 (5.2)	
Smoking status, n (%)											
Non-smoker	41 (61.2)	4 (57.1)	15 (75.0)	0.555[a]	–	1 (50.0)	59 (64.1)	0.999	13 (76.5)	47 (61.0)	0.276[b]
Smoker	26 (38.8)	3 (42.9)	5 (25.0)			1 (50.0)	33 (35.9)		4 (23.5)	30 (39.0)	
Comorbidities											
Negative	53 (79.1)	4 (57.1)	12 (60.0)	0.142[a]	–	1 (50.0)	68 (73.9)	0.463	13 (76.5)	56 (72.7)	0.752[b]
Positive	14 (20.9)	3 (42.9)	8 (40.0)		–	1 (50.0)	24 (26.1)		4 (23.5)	21 (27.3)	
Total	67 (100.0)	7 (100.0)	20 (100.0)		–	2 (100.0)	92 (100.0)		17 (100.0)	77 (100.0)	
Comorbidities*, n (%)											
Cardiovascular disease	6 (8.1)	3 (30.0)	2 (8.0)	0.007[a]	–	1 (33.3)	10 (9.4)	0.015[a]	1 (5.3)	10 (11.1)	0.680[b]
Rheumatic disease	2 (2.7)	–	–		–	–	2 (1.9)		–	2 (2.2)	
Endocrine disease	7 (9.5)	–	2 (8.0)		–	1 (33.3)	8 (7.5)		1 (5.3)	8 (8.9)	
Autoimmune disease	1 (1.4)	–	2 (8.0)		–	–	3 (2.8)		1 (5.3)	2 (2.2)	
Malignancy		–	3 (12.0)		–	–	3 (2.8)		1 (5.3)	2 (2.2)	
Other diseases**	58 (78.4)	7 (70.0)	16 (64.0)		–	1 (33.3)	80 (75.5)		15 (78.9)	66 (73.3)	
Side effects, n (%)											
None	45 (67.2)	4 (57.1)	10 (50.0)	0.381[a]	–	1 (33.3)	66 (70.2)	0.256[a]	14 (82.4)	53 (66.3)	0.222[b]
Any local SEs	12 (17.9)	2 (28.6)	6 (30.0)		–	1 (33.3)	12 (12.8)		–	13 (16.3)	
Any systemic SEs	12 (17.9)	2 (28.6)	7 (35.0)		–	1 (33.3)	16 (17.0)		3 (17.6)	14 (17.5)	

BMI: body mass index; SE: side effect. [a]Fisher–Freeman–Halton test; [b]Pearson's χ^2 test. *Multiple response variables. Among the participants, 69 had none, 18 had only one, and seven had more than one coexisting condition. **Other diseases include uveitis, seborrheic dermatitis, endometriosis, asthma, and migraine.

N501Y), as previously reported[7-9,16]. In Brazil, where the P.1 variant is widely circulated, the estimated efficacy of CoronaVac was reported to be 49.6% after at least one dose, and another phase 4 study similarly reported 50.7%[15,16]. In another study, it was also reported that antibodies from naturally infected or CoronaVac-vaccinated individuals were less effective at neutralizing P.1 isolates[8].

As previously reported, anti-spike IgG (innate immunity) maintains for at least 8 months after COVID-19[18]. However, there are still evidence gaps for the duration of vaccine-induced immunity, appropriate timing for booster shots, and interchangeability of vaccines, all of which need to be assessed in further studies.

The seropositivity was predominantly detected in 31–45 years of the age group (55.4%), normal BMI (47.8%), non-smokers (64.1%), those without any comorbidity (73.9%), and those without any SEs (70.2%). Although there are insufficient data on the relationship between antibody responses and demographic and clinical variables, it has been reported in detail that the efficacy of the vaccine in the elderly and young adults is similar, and the elderly have lower neutralizing antibody titers[6,15,19]. The real-life data in Chile also reported that the efficacy of the vaccine in fully immunized persons aged 60 years or above was 66.6% (65.9% in 16–59 years of age group) for the prevention of COVID-19, 85.3% (87.5% in 16–59 years of age group) for the prevention of hospitalization, 89.2% (90.3% in 16–59 years of age group) for the prevention of intensive care unit admission, and 86.5% (86.3% in 16–59 years of age group) for the prevention of COVID-19-related death[5]. The seropositivity among HCWs with obesity was low (7.6%), similar to a study with mRNA vaccine[20]. This lower seropositivity can be due to the fact that obesity induces defects in B cells and compromised immune system responds poorly to vaccination against influenza, rabies, tetanus, and hepatitis B[21]. Smoking is associated with numerous diseases and impacts both innate and adaptive immunity. The lower rate of antibody response in smokers (35.9%) was compatible with the data previously reported; smoking reduces avidity and/or synthesis of Ig (IgM, IgA, IgG) in B cells[20-23]. The antibody response was lower (40%) among participants with any comorbidity, similar to the efficacy report of the phase 3 clinical trial (48.9%) in Brazil[15]. As previously reported, the defects in blood glucose regulation due to diabetes can cause dysfunction in cellular and humoral immunity, thus explaining the lower seropositivity among diabetic participants[24].

In our study, the frequencies of overall SEs after vaccinations were in agreement with the phase 1/2 clinical trials in China[13] (29% and 33%, respectively) but higher than the phase 3 clinical trials in Turkey[14] (18.9%). The most common SE (local pain at the injection site), distribution (local pain at injection site, headache, fatigue, and myalgia), and severity of all SEs were similar to the findings in clinical phase trials[13-15].

Our study has some limitations: humoral immunity was tested semiquantitatively, and neutralizing activity or vaccine-induced cellular immunity was not studied. The duration

Table 3. Side effects within seven days after each dose of CoronaVac.

Side effects within seven days	After the first dose of vaccine n (%)	After the second dose of vaccine n (%)
None	59 (48.8)	67 (59.8)
Localized pain	19 (15.7)	13 (11.6)
Localized redness	–	1 (0.9)
Localized swelling	2 (1.7)	1 (0.9)
Weakness	10 (8.2)	4 (3.6)
Headache	13 (10.7)	12 (10.7)
Fever	–	1 (0.9)
Myalgia	5 (4.1)	5 (4.4)
Dyspnea	1 (0.8)	–
Nausea–vomiting	3 (2.5)	1 (0.9)
Arthralgia	3 (2.5)	3 (2.7)
Hypertension	2 (1.7)	2 (1.8)
Runny nose	2 (1.7)	1 (0.9)
Asthenia	1 (0.8)	1 (0.9)
Numbness, tingling in the left arm	1 (0.8)	–

of antibody protection and reinfection rates after vaccination remain to be identified. This study was conducted in a small group of HCWs.

CONCLUSIONS

We found that vaccination by two-dose CoronaVac elicits a specific humoral response, and it was well tolerated as all SEs experienced were mild and moderate in severity. However, studies with larger numbers of populations and longer follow-up periods will be beneficial in terms of determining the duration of immunity and late SEs. Continuously following up the current variants in circulation in both vaccinated and unvaccinated populations by genome analyzes and monitoring of vaccine-induced immunity are crucial to develop new control measures and to determine the timing of new vaccination strategies.

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AUTHORS' CONTRIBUTIONS

HHG: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **IÖ:** Formal Analysis, Investigation, Writing – original draft. **HEA:** Formal Analysis, Writing – review & editing. **AK:** Data curation. **SK:** Data curation. **MÖ:** Writing – review & editing. **EG:** Formal Analysis.

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ORIGINAL ARTICLE
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Contribution of aspiration to the diagnosis of lung cancer in endobronchial ultrasound-guided fine-needle biopsy

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SUMMARY

OBJECTIVE: Endobronchial ultrasound-guided transbronchial needle aspiration has been successfully applied in both diagnosis and staging of mediastinal and hilar lymphadenopathies and masses, especially in malignant cases. However, the optimal procedure of Endobronchial ultrasound-guided transbronchial needle aspiration to further increase diagnostic yield and minimize processing complexity remains controversial. This study aims to compare aspiration biopsy (Endobronchial ultrasound-guided transbronchial needle aspiration) and non-aspiration biopsy (Endobronchial ultrasound-guided transbronchial needle capillary sampling) in terms of sample adequacy, diagnosis, and quality in malignant cases.

METHODS: Between March 2018 and June 2020, Endobronchial ultrasound-guided was performed sequentially on patients with mediastinal and/or hilar lymph nodes that were considered malignant. Each lymphadenopathy was sampled with and without aspiration. A single-blinded pathologist evaluated the samples.

RESULTS: A total of 84 lymph nodes evaluations of 51 patients were included. Most samples were taken from the right lower paratracheal lymph nodes (n=27, 32.2%) and subcarinal LN (n=21, 25%). The mean size of the lymph nodes was 21.21±8.257 (8–40) mm. The agreement between the two procedures in terms of sample adequacy and diagnostic yield was 69.1% (95%CI 58–78.7, p=0.076). In addition, according to the goodness-of-fit statistics, the kappa values were 0.255 (p=0.015) and 0.302 (p=0.004) for sample adequacy and diagnostic yield, respectively. There was no difference between the two procedures in relation to complications.

CONCLUSION: Although the agreement between the two procedures is weak, Endobronchial ultrasound-guided transbronchial needle capillary sampling can be performed with less personnel, without reducing diagnostic yield and tissue adequacy. These findings can assist clinicians in determining the optimal procedure for Endobronchial ultrasound-guided.

KEYWORDS: Lung cancer. Fine needle biopsy. Bronchoscopy. Lymph node biopsy, sentinel.

INTRODUCTION

Clinical studies have shown that endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is similar to or even better than surgical mediastinoscopy in the diagnosis of mediastinal and hilar lymphadenopathies, possibly due to its low cost and safe diagnostic technology[1]. EBUS-TBNA has gained more importance since it was adopted for use in staging, especially in malignant cases. In the traditional EBUS-TBNA procedure, aspiration is applied following the removal of the metal stylet from the inner lumen of the needle after entering

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the lesion. Nevertheless, the optimal procedure of EBUS-TBNA remains controversial[2-4]. Fine-needle sampling without the use of aspiration, i.e., capillary sampling, has been investigated in various tissues (e.g., breast, thyroid, and peripheral lymph nodes [LNs]) and shown to have similar diagnostic yield and cause less trauma compared to fine-needle aspiration[5,6]. However, the use of EBUS-guided needle capillary sampling (EBUS-TBNCS) has been described only in a limited number of studies[7-11].

Since there is considerable debate whether aspiration is required during EBUS-TBNA, we conducted a prospective, randomized controlled study to determine the optimal EBUS-TBNA procedure for detecting mediastinal and hilar lymphadenopathies in malignant cases. In this study, we aimed to compare the EBUS-TBNA and EBUS-TBNCS procedures in terms of sample adequacy, diagnosis, and quality in malignant mediastinal and hilar LNs.

METHODS

The study was carried out between March 2018 and June 2020 at the Department of Chest Diseases of Inonu University Turgut Özal Medical Center. Approval was obtained from the interventional ethics committee of the university for this prospective, randomized, interventional, single-blind study (approval number: 2018/107). Detailed information was given to the patients before the procedure, and all signed a consent form.

Patient population

All patients who presented to our clinic with a mediastinal mass, mediastinal LN, or parenchymal mass invading the mediastinum and who were considered to be clinically and radiologically malignant underwent EBUS sequentially. Exclusion criteria included age <18 years, exclusions for EBUS-TBNA (uncontrolled coagulopathy or bleeding diathesis, clinical stability), benign pathological results, and non-informed consent.

Study design

The volunteers' demographic data (age, gender, and comorbidities), anamnesis, smoking history, results of computed tomography (CT), results of positron emission tomography (PET)-CT if undertaken, complications that developed during the procedure, the number of LNs sampled, and pathological results were documented. The biopsy procedure was performed from the same lesion under the same conditions with and without aspiration. To prevent the first pass effect, biopsies with and without aspiration were performed in each LN in a different order. The samples were coded with numbers. The researcher was the only person who had knowledge of the code of each sample. The pathologist was not provided this information to ensure that the study was conducted in a single-blinded manner (Figure 1).

Imaging was performed with a fiberoptic bronchoscope (Fujinon Fujifilm Ultrasonic Processor SU-1), and samples were taken. A 22G fenestrated needle (Cook Medical, USA) was used as the aspiration needle.

To summarize the technique, during the procedure, before the bronchoscope and catheter needle were removed from their sheath, they were directed to the targeted point, and the metal tip of the catheter was allowed to contact the mucosa. In this position, the needle was inserted quickly and hard. The penetration of the needle through the wall along its entire length was checked with an ultrasound image. For this purpose, the bronchoscope was pushed through the catheter and advanced toward the bronchial wall. When the needle was in the targeted place, the artifacts were removed with the stylet. Both methods were the same up to this point. Then, the stylet was removed and aspirated with negative pressure using a 10-mL injector for aspiration method. In the non-aspirated biopsy method, after using a stylet to remove the artifacts from the needle, the stylet was pulled back by 10 cm and the needle was advanced back and forth inside the LN without aspiration, and the biopsy sample was obtained. In both methods, the back-and-forth movements were performed in the LN for 25–30 times.

Statistical analysis

The variables used in analyses were summarized as number (percentage) and mean±standard deviation/median (min–max) values according to their qualitative and quantitative nature. Fisher's exact and chi-square tests were used to determine the statistically significance/relationship between the groups of qualitative variables. The significance of differences between the non-aspirated and aspirated biopsy methods was analyzed using the McNemar–Bowker test. The degree of agreement between these two methods was evaluated using the kappa statistics. In addition, the diagnostic performance of the reference test was measured based on accuracy, sensitivity, and selectivity among the diagnostic test criteria. A $p<0.05$ was accepted as the level of significance. IBM SPSS Statistics version 26.0 was used to perform statistical analyses.

RESULTS

A total of 84 LN evaluations of 51 patients were included in the study. The demographic data of the patients and biopsied LNs are summarized in Table 1. Notably, 11 (21.6%) patients were females and 40 (78.4%) were males.

Two LN aspirations were performed in most of the patients. The number of LN aspirations performed was 2 of 27, 1 of 21,

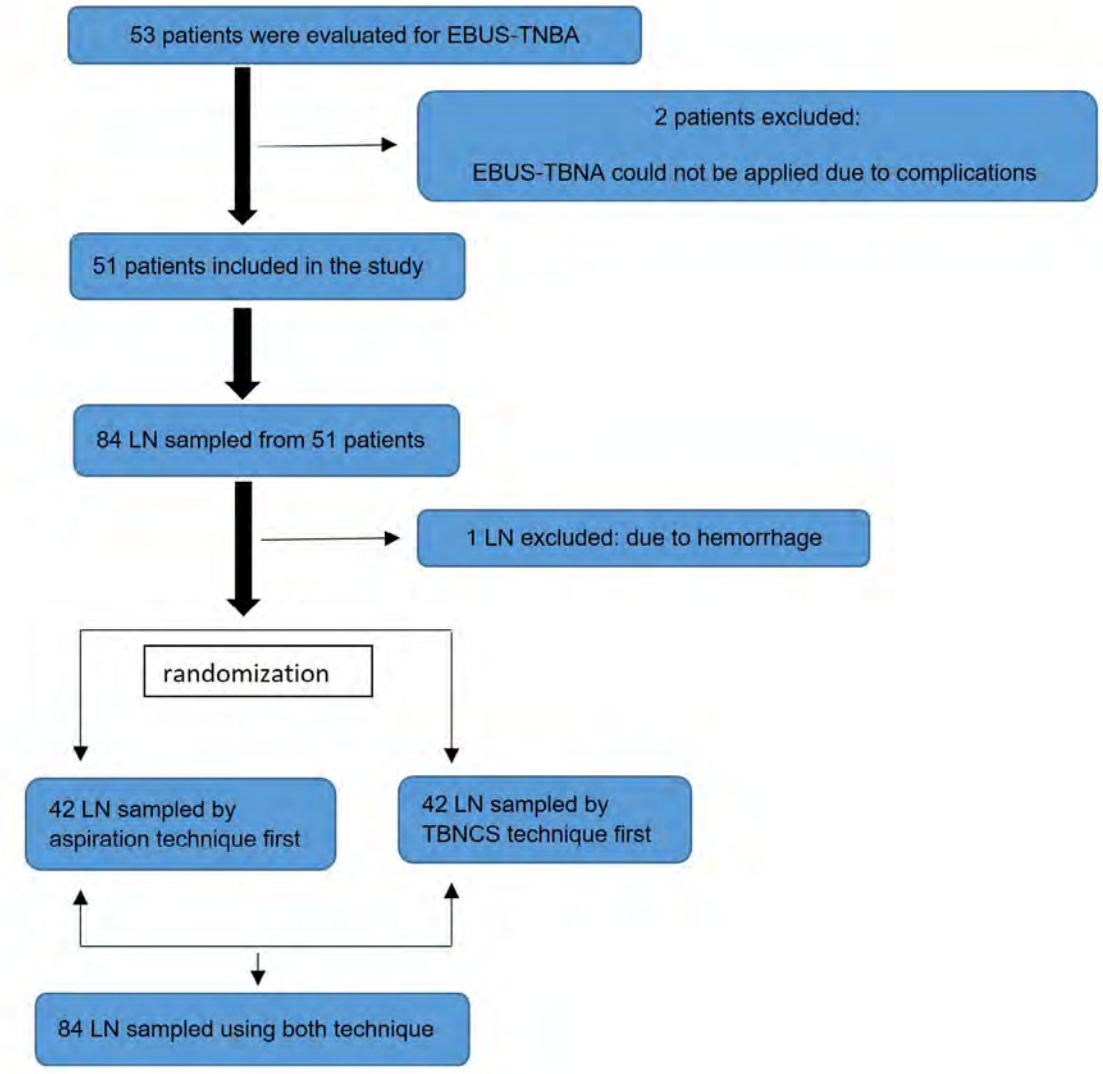

Figure 1. EBUS-TBNA: endobronchial ultrasound-guided transbronchial needle aspiration; LN: lymph node; TBNCS: transbronchial needle capillary sampling. Study flowchart of enrolled patients.

and 3 of 3 patients. Aspiration biopsies were mostly performed from the right lower paratracheal LN (n=27, 32.2%), followed by subcarinal LN (n=21, 25%) and left and right lobar LNs (n=2, 2.4% and n=4, 4.8%, respectively). The mean size of the LNs was 21.21±8.257 (8–40) mm.

Agreement on sample adequacy and diagnostic yield

The agreement between the EBUS-TBNA and EBUS-TBNCS procedures was high, with no significant difference in the sample yield. The agreement rate for sample adequacy was 69.1% (95%CI, 58–78.7, p=0.076). In addition, according to the goodness-of-fit statistics, the kappa value was 0.255 (p=0.015). The agreement rate for diagnostic yield was 69.1% (95%CI, 58–78.7, p=0.076). The kappa value for diagnostic yield was 0.302 (p=0.004) (Table 2).

Considering the subtypes of the pathological diagnoses, squamous cell carcinoma was present in 19 (37.2%) patients, small cell lung carcinoma in 14 (27.4%), adenocarcinoma in 13 (25.4), and leukemia, lymphoma, and metastasis in 1 (2%). One (2%) patient was suspected for malignancy, and in 1 of 2 patients who could not be subtyped by EBUS, the postoperative histology was reported as small cell lung cancer, and the final results were unknown since the other patient did not

Table 1. Demographic data and characteristics of biopsied lymph nodes.

	n (%)
Age (mean), years	61.6±10.3
Gender	
Female	11 (21.6)
Male	40 (78.4)
Smoking status	
Active smoker	20 (39.2)
Tobacco-naive	15 (29.4)
Former smoker	16 (31.4)
Referring department	
Neurosurgery	3 (5.9)
Internal diseases	1 (2.0)
Thoracic surgery	4 (7.8)
Chest diseases	39 (76.5)
Gynecology	1 (2.0)
Cardiology	1 (2.0)
Oncology	2 (3.9)
Comorbidities	
Hypertension	9 (17.6)
Diabetes mellitus	7 (13.7)
Coronary artery disease	8 (15.6)
Malignancy	3 (5.8)
Chronic obstructive pulmonary disease	3 (5.8)
Asthma	1 (2.0)
Hypothyroidism	1 (2.0)
Complications	
Minimal hemorrhage	6 (11.8)
Desaturation	3 (5.9)
Hypertension	1 (2.0)
Desaturation and minimal hemorrhage	1 (2.0)
Biopsied lymph nodes	
Lymph node size, mm	84
Lymph nodes by location	21.21±8.257
4L	9 (10.7)
4R	27 (32.1)
7	21 (25)
10L	2 (2.4)
10R	6 (7.1)
11L	6 (7.1)
11R	7 (8.3)
12L	2 (2.4)
12R	4 (4.8)
Malignancy subtype	
Squamous cell lung carcinoma	19 (37.2)
Small cell lung carcinoma	14 (27.4)
Lung adenocarcinoma	13 (25.4)
Large cell lung carcinoma	2 (2.0)
Leukemia	2 (2.0)
Lymphoma	2 (2.0)
Metastasis	2 (2.0)
Suspicious for malignancy	2 (2.0)

present to our center again and could not be reached by phone. The operation result of the patient who had been diagnosed with non-small cell lung carcinoma was reported as large cell lung carcinoma.

There were no life-threatening complications among the EBUS patients. While minimal complications were observed in 11 (21.6%) patients, no complication developed in 40 (78.4%) patients. In two patients, due to hypertension and desaturation before the procedure, the procedure could not be initiated. The data of these patients were not included in the study statistics. A further patient had severe hemorrhage during the procedure; thus, after aspiration biopsy, the procedure was terminated without performing non-aspiration biopsy. The hemorrhage was controlled with cold saline solution, and no other complication occurred. The result of the aspiration biopsy of this patient was consistent with vasculitis, and the patient was excluded from the study. A total of 11 (21.6%) patients had minimal complications that did not require the termination of the procedure (Table 3). Minimal complications seen were desaturation (n=3, 5.9%), hypertension (n=1, 2%), minimal hemorrhage (n=6, 11.8%), and desaturation and hemorrhage (n=1, 2%).

Although there were no serious and mortal complications in the patients, the relationship between minimal complications and comorbidities was evaluated, and there was no significant relationship (p>0.05) (Table 3).

DISCUSSION

Our study showed weak agreement between conventional aspiration biopsy performed by EBUS-TBNA and capillary biopsy (EBUS-TBNCS) in terms of cytological sample adequacy of malignant LNs and diagnostic yield in patients with lung cancer. A capillary biopsy can be preferred in the EBUS procedure undertaken in malignant cases since it has similar sample adequacy, low risk of bleeding, and a short procedure time although the last parameter was not evaluated in our study. Although more data and further studies are needed, our findings may be a guide for physicians performing interventional procedures in determining the optimal EBUS-TBNA biopsy procedure.

Since first described by Paget in 1853 (on a breast tumor), fine-needle aspiration has been widely used[12]. However, over time, the role of aspiration in fine-needle biopsy has been discussed. Zajdela et al. retrospectively compared 635 capillary sampling and 7,877 fine-needle aspiration in breast tumors and found no difference in diagnostic yield or cellularity, and reported less blood in samples obtained without aspiration[13]. Mair et al. found no difference in diagnostic yield between conventional fine-needle aspiration and fine-needle capillary

Table 2. Agreement between EBUS-TBNA and EBUS-TBNCS in terms of sample adequacy and diagnostic yield for the biopsied lymph nodes (n=84).

	EBUS-TBNA			
EBUS-TBNCS	Sample adequacy (%)	Negative	Positive	Agreement (95%CI)
	Negative	11 (14)	18 (21)	69.1 (58–78.6)
	Positive	8 (9)	47 (56)	
	Diagnostic yield (%)			
	Negative	14 (17)	18 (21)	
	Positive	8 (10)	44 (52)	

EBUS-TBNA: endobronchial ultrasound-guided transbronchial needle aspiration; EBUS-TBNCS: endobronchial ultrasound-guided needle capillary sampling; CI: confidence interval.

Table 3. Relationship between complications during the procedure and comorbidities of the patients.

		Complication		Total, n (%)	p-value*
		Absent, n (%)	Present, n (%)		
Hypertension	Absent	33 (82.50)	9 (81.82)	42 (82.35)	0.999
	Present	7 (17.50)	2 (18.18)	9 (17.65)	
	Total	40 (100.00)	11 (100.00)	51 (100.00)	
Diabetes mellitus	Absent	34 (85.00)	10 (90.91)	44 (86.27)	0.999
	Present	6 (15.00)	1 (9.09)	7 (13.73)	
	Total	40 (100.00)	11 (100.00)	51 (100.00)	
CAD	Absent	33 (82.50)	10 (90.91)	43 (84.31)	0.668
	Present	7 (17.50)	1 (9.09)	8 (15.69)	
	Total	40 (100.00)	11 (100.00)	51 (100.00)	
COPD	Absent	38 (95.00)	10 (90.91)	48 (94.12)	0.526
	Present	2 (5.00)	1 (9.09)	3 (5.88)	
	Total	40 (100.00)	11 (100.00)	51 (100.00)	
Malignancy	Absent	37 (92.50)	11 (100.00)	48 (94.12)	0.999
	Present	3 (7.50)	0 (0.00)	3 (5.88)	
	Total	40 (100.00)	11 (100.00)	51 (100.00)	
Asthma	Absent	39 (97.50)	11 (100.00)	50 (98.04)	0.999
	Present	1 (2.50)	0 (0.00)	1 (1.96)	
	Total	40 (100.00)	11 (100.00)	51 (100.00)	
Hypothyroidism	Absent	39 (97.50)	11 (100.00)	50 (98.00)	0.999
	Present	1 (2.50)	0 (0.00)	1 (2.00)	
	Total	40 (100.00)	11 (100.00)	51 (100.00)	

CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease.*Fisher's exact test.

sampling in a study of 100 superficial masses in various body regions, and they also showed that the samples obtained using capillary sampling had better quality[6]. In our study, although there was a weak agreement rate between EBUS-TBNA and EBUS-TBNCS, the latter did not yield samples of superior quality compared to the aspiration method. In a recent prospective EBUS study conducted with 66 patients, Boonsarngsuk et al. compared three different aspiration pressure levels (0 [no suction], -20, and -40 cm H_2O) and determined that the diagnostic value of biopsies performed without aspiration was lower (63.6, 75.8, and 83.3%, respectively)[7]. Casal et al., prospectively comparing biopsies with and without aspiration in 115 patients with a total of 192 LNs, reported no difference between sample quality and diagnosis rate and noted that there

was a high agreement between the two methods[9]. Similarly, in a prospective study of 38 patients, Rodriguez et al. found a high agreement between aspiration and non-aspiration biopsies (sample adequacy: 95.5%; diagnosis specificity: 84%)[8]. Harris et al. compared the same two methods in a prospective non-inferiority study of 24 patients and observed no difference[14]. Although there was weak agreement in our study, as the authors, we think that TBNCS can be used because of its ease of procedure, less personnel requirement, time saving, and no complications.

Complications in EBUS-TBNA are extremely rare. The most common complication is bleeding, and other rare complications include infection, pneumothorax, and device or needle damage. In a study undertaken by the Japan Respiratory Endoscopy Society covering a total of 7,345 cases reported from 210 centers, the complication rate was only 1.23%. The most common complication was bleeding (n=50), followed by infection (n=14), ultrasound bronchoscope damage (n=98), and needle damage (n=15)[15]. Similarly, another systematic review of adverse events in 16,181 patients undergoing endosonography for mediastinal and hilar LNs or central lung masses reported only 23 (0.14%) serious adverse events without mortality[16]. In our study, in one patient, due to severe hemorrhage during the procedure, after aspiration biopsy, the procedure was terminated without performing non-aspiration biopsy. Our study showed that in accordance with the literature, EBUS-TBNA is a generally safe method, and different EBUS-TBNA procedures have a similar probability of complications.

The main limitations of our study are that it was conducted in a single center and there was a single operator. A multicenter study would provide better data to confirm the statistical significance of the results obtained using different EBUS-TBNA procedures. Another limitation may be related to the number of times the needle was moved within the LN. Although there is no evidence for the ideal number, it can be argued that this number of back-and-forth movements was high, which could have led to more bloody samples. In addition, one needle was used for each patient, and different needles were not used for different methods in the same patient. Using a separate needle for each LN in the same patient and different needles for the two methods may have been ideal to prevent contamination; however, we were not able to do this due to financial reasons. However, to minimize the risk of contamination and provide randomization, we changed the order of methods performed in our patients.

CONCLUSIONS

EBUS-TBNA requires the manual aspiration of lock-mechanism syringe or additional personnel to perform aspiration during biopsy. This requirement can be eliminated with the use of the EBUS-TBNCS technique. According to the results of our study, we recommend EBUS-TBNCS technique as a simpler and equally effective technique for EBUS-guided biopsies of mediastinal and hilar LNs in the diagnosis of patients with suspected malignancy.

AUTHORS' CONTRIBUTIONS

ANG: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. MY: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **ANA:** Investigation, Methodology, Resources, Formal Analysis, Writing – original draft, Writing – review & editing. **AKA:** Data curation, Formal Analysis, Methodology, Resources, Writing – original draft.

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Magnetic resonance imaging evaluation of incidentally detected hyperechoic liver lesions: comparison of two modalities in terms of detection, diagnosis, and morphological features

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SUMMARY

OBJECTIVE: This study aimed to investigate and compare the ultrasonography and contrast-enhanced magnetic resonance imaging characteristics of incidentally detected hyperechoic focal liver lesions.

METHODS: Seventy-four patients (29 males and 45 females) who had undergone a B-mode ultrasonography and contrast-enhanced magnetic resonance imaging examination were included in this study. A total of 91 hyperechoic lesions detected on ultrasonography were evaluated. The ultrasonography features of these hyperechoic lesions were recorded, and the results were compared with those acquired from contrast-enhanced magnetic resonance imaging. The results were compared statistically using the Shapiro-Wilk, McNemar, and Wilcoxon signed-rank tests.

RESULTS: A corresponding lesion was found on contrast-enhanced magnetic resonance imaging in 72 of the 91 (79.1%) hyperechoic lesions detected on ultrasonography. Forty-one (56.9%) of the magnetic resonance imaging-defined lesions were typical hemangiomas, while 10 (13.9%) were focal steatosis areas and 4 (5.6%) were diagnosed with hepatocellular carcinoma. In contrast, 6 lesions (8.3%) were diagnosed as simple hepatic cysts, 4 (5.6%) as sclerosing hemangioma, 2 (2.8%) as thrombosed hemangioma, 1 (1.4%) as focal nodular hyperplasia, 1 (1.4%) as hamartoma, 2 (2.8%) as hydatid cysts, and 1 (1.4%) as hepatic lipoma. No statistically significant differences were found between ultrasonography and magnetic resonance imaging in terms of the segmental classification of the true positive lesions based on contour structures and lesion area measurements (p=0.558, p=0.375, and p=0.636, respectively).

CONCLUSIONS: Incidentally detected hyperechoic zones may not necessarily be detected on magnetic resonance imaging. This may be secondary to focal hepatic steatosis or false interpretation of the radiologist. Lesions requiring therapy must be considered in the differential diagnosis.

KEYWORDS: Ultrasound imaging. Incidental finding. Hemangioma. Liver steatosis. Magnetic resonance imaging.

INTRODUCTION

Advancements in technical ultrasonography (US) standards and the increasing number of abdominal US examinations performed globally have also led to an increase in the number of incidentally detected hyperechoic liver lesions on US. These lesions sometimes create confusion in the differential diagnosis and necessitate further imaging examinations, such as magnetic resonance imaging (MRI). US echogenicity increases

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in many instances, including the presence of steatosis, calcification, fibrosis, and gas[1]. Benign lesions, such as hemangioma, focal steatosis, lipoma, hamartoma, and focal fibrosis, as well as malignant lesions, e.g., primary and secondary malignancies, are considered in the differential diagnosis of hyperechoic liver lesions[2,3]. This study presents the results of the dynamic MRI examinations of focal hyperechoic liver lesions detected incidentally on US.

METHODS

Patient selection and evaluation

This retrospective study was approved by our institutional ethical committee and was carried out in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines. Notably, 74 patients who had undergone a B-mode US and dynamic MRI examination of the liver were included in this study. These patients who had undergone a US examination for various reasons were incidentally detected to have hyperechoic lesions. Patients with known liver tumors, with extrahepatic malignancies with the potential to metastasize to the liver, with a chronic liver disease such as cirrhosis, and with an interval of 60 days or longer between the US and MRI examinations were excluded from this study. The MRI examinations of the patients whose focal hyperechoic liver lesions were incidentally detected on US were evaluated by two expert radiologists with 16 and 14 years of experience in their profession. The results were obtained based on consensus. Both radiologists evaluated the dynamic MRI images to define the characteristics of the lesions and diagnose them in light of clinical data and laboratory test results. The radiologists who evaluated the MRI studies had no information about the size, location, and other characteristic properties of the lesions detected on US.

Sonographic evaluations

The US examinations of all patients were performed using a Philips Epiq 7G device (Andover, MA, USA) with an abdominal transducer. No sonographic contrast material was utilized. The patients underwent their US examinations following a fasting period of 6 h. The US examinations were performed during deep inspiration, with the patients holding their breath. Certain features of hyperechoic liver lesions detected on US, such as the largest diameter, segmental location, contour properties, and compression characteristics of adjacent structures, were documented. In patients with more than one lesion, the data of each lesion were recorded separately according to its segmental location.

Magnetic resonance imaging evaluations

All MRI examinations were undertaken with a 1.5 Tesla scanner (Ingenia; Philips Healthcare, The Netherlands). The following sequences were obtained for all patients: axial and coronal balanced turbo field echo T2-weighted (T2W), axial spectral attenuated inversion recovery T2W, diffusion-weighted imaging, ADC mapping, and dual-echo fast field echo (FFE). In addition, the T1-weighted (T1W) fat-suppressed gradient echo thrive sequence was utilized in all patients both with and without intravenous gadolinium administration (0.1 mmoL/kg body weight). Following the gadolinium injection, contrast-enhanced images were acquired at the arterial, portal, and venous phases. The acquisition parameters of the dual-echo sequence were as follows: field of view (FOV), 450×398 mm; matrix size, 280×248; slice thickness, 5 mm; flip angle, 75°; repetition time (TR), 106 ms; in-phase echo time (TE), 4.6 ms; and out-of-phase TE, 2.3 ms. The acquisition parameters of the dynamic MRI sequence were selected as follows: FOV, 450×401 mm; matrix size, 300×229; slice thickness, 6 mm; flip angle, 10°; TR, 4.2 ms; and TE, 2.1 ms.

An ovoid or geographical lesion located adjacent to the gallbladder fossa, falciform ligament, or capsule with no mass effect, appearing as hyperintense in the in-phase image and hypointense in the out-of-phase image on the dual-echo FFE sequence, was defined as an area of focal fatty infiltration. Focal lesions with the following MRI characteristics were defined as hemangiomas: hypointense in the T1W images, hyperintense in the T2W images, peripheral nodular contrast enhancement in the arterial dynamic phase, and centripetal contrast enhancement in the portal and late venous phases. Hyperintensity in the T2W sequence, peripheral nodular contrast enhancement starting in the arterial phase, and hypointense areas in the portal and late phases were interpreted to indicate thrombosed hemangiomas. T2W hyperintensity with mild peripheral nodular contrast enhancement in the late phase and capsular contraction was defined as a sclerosing hemangioma. Lesions demonstrating T1W hypointensity, T2W mild hyperintensity, hyperintensity in the dynamic arterial phase, and iso-intensity or mild hyperintensity in the portal and late venous phases were defined as focal nodular hyperplasia. T1W hypointense and T2W substantially hyperintense lesions showing no contrast enhancement at the dynamic phases were defined as simple hepatic cysts. T1W hypointense lesions showing substantial hyperintensity in the T2W sequence and thin peripheral contrast enhancement in the dynamic series were diagnosed as hamartomatous cysts. Lesions appearing as hypointense in T1W and hyperintense in T2W sequences and possessing a peripheral hypointense rim

that demonstrated contrast enhancement in the late phase and had curvilinear internal structures were interpreted as hydatid cysts. Lesions that appeared as hypointense foci in T1W and moderately hyperintense foci in T2W sequences and demonstrated hyperenhancement in the dynamic arterial phase and a quick washout following the arterial phase were diagnosed as hepatocellular carcinomas (HCCs). Lesions demonstrating hyperintensity in both T1W and T2W sequences, hypointensity in fat-saturated T2 sequences, and a hypointense band of India ink artifact in the out-of-phase sequence were considered as hepatic lipomas.

The dimensions, contour characteristics, and segmental localization of the lesions were documented from the MRI images. All the lesions were evaluated using the MRI sequence where they were most apparent. Contrast-enhanced dynamic imaging was performed for hemangiomas and HCCs, whereas T2W sequences were obtained for simple and hydatid cysts. Multiple measurements were obtained, and the longest diameter was noted. In patients with more than one lesion, the results of each lesion were recorded separately. Lesions that could not be categorized according to the MRI characteristics were excluded from this study.

Statistical analysis

SPSS version 21.0 was used for the statistical analysis of the data. Categorical variables were recorded as numbers and percentages. The Shapiro-Wilk test was utilized for the spatial evaluation of numeric parameters. Variables showing a normal distribution were defined with the mean and standard deviation parameters, while those not demonstrating a normal distribution were expressed as median, minimum, and maximum values. The McNemar test was conducted for the comparison of categorical data obtained from MRI and US, whereas the Wilcoxon signed-rank test was performed for the comparison of nonparametric numerical data.

RESULTS

Twenty-nine (39.2%) of 74 patients included in this study were males and 45 (60.8%) were females. The mean age of the patients was 45.58±14.06 years. The interval time between the US and MRI examinations of the patients ranged from 0–55 days, with a median value of three days. On US, a total of 103 lesions were detected, of which 12 could not be categorized on MRI, and thus they were excluded from this study. The US and MRI findings of the 91 lesions detected on US were compared.

For 72 (79.1%) of the 91 hyperechoic lesions detected on US, a corresponding lesion was detected on MRI. No corresponding lesion was identified on MRI in the remaining 19 lesions. No statistically significant difference was present between the segmental localizations of the sonographic and MRI positive lesions that were defined on both US and MRI (p=0.558). Only five (5.49%) of the 91 lesions were the segments of localizations differed according to the diagnostic modality (US or MRI).

Forty-one (56.9%) of the MRI-defined lesions were typical hemangiomas, whereas 10 (13.9%) were focal steatosis areas. Four (5.6%) lesions were diagnosed as HCCs. The diagnostic categorization of the MRI-defined lesions is demonstrated in Table 1.

Sixty-seven (73.6%) of the US-diagnosed 91 lesions were defined as regularly contoured and 24 (26.4%) as irregularly contoured. The MRI numbers corresponding to these US numbers were 53 (73.6%) and 19 (26.4%), respectively, in the 72 lesions detected at MRI. No statistically significant difference was found between US and MRI in terms of the classification of the lesions according to their contour characteristics (p=0.375).

Area calculations were performed by the proper multiplication of the long and short axes of the lesions. The results of these lesion measurements were recorded as minimum 37.5 mm^2, maximum 4.928 mm^2, and median 251 mm^2 for US, and minimum 42 mm^2, maximum 4420 mm^2, and median 226.5 mm^2 for MRI. There was no statistically significant difference between the US and MRI results in terms of lesion area measurements (p=0.636).

DISCUSSION

The incidental finding of a focal hyperechoic lesion on US is a frequently encountered situation, and a proper differential

Table 1. Diagnosis of the lesions according to MRI findings.

Diagnosis	Number	(%)
Typical hemangioma	41	56.9
Sclerosing hemangioma	4	5.6
Thrombosed hemangioma	2	2.8
Focal nodular hyperplasia	1	1.4
Focal steatosis	10	13.9
Hamartoma	1	1.4
Hepatocellular carcinoma	4	5.6
Simple hepatic cyst	6	8.3
Hydatid cyst	2	2.8
Hepatic lipoma	1	1.4
Total	72	100

MRI: magnetic resonance imaging.

diagnosis is not always possible by US alone. The literature indicates that these focal hyperechoic lesions mostly represent a hemangioma[4]. However, the differential diagnosis list also includes various other lesions, most of which are benign. Although US and MRI have different advantages and disadvantages in detecting lesions, MRI plays a problem-solving role in these situations.

In recent studies, it has been shown that MRI is superior to the detection of focal liver lesions compared with US and computed tomography due to its high soft tissue resolution[5]. To the best of our knowledge, there is no study in the English language literature assessing the sensitivity of MRI in the detection of incidentally detected hyperechoic lesions. In this study, MRI was not able to detect 19 of the 91 focal hyperechoic lesions (20.8 %) observed during US examinations. This may be due to the interpretation error of the radiologist who performed the US examination. Another explanation may be that these lesions might be visible during US examinations due to the slight sonographic contrast created by the focal lesion over the liver parenchyma, but their MRI signals might not be strong enough to create MRI contrast sufficient for visualization (Figure 1). In addition, focal fatty infiltration may be associated with increased hepatic iron accumulation and can hamper the detection of focal fatty infiltration[5].

The gold standard for the detection of liver steatosis is a biopsy[6], but due to its invasive nature, hepatic steatosis is usually screened by US to perform a qualitative evaluation[7]. In contrast, qualitative and quantitative evaluations are undertaken with the utilization of certain MRI sequences[8]. It has been reported that MRI has 76.7–90% sensitivity and 87.1–91% specificity in the imaging diagnosis of hepatic steatosis[9-12]. Opposed-phase imaging performed within the dual-echo sequence in MRI can make the proper diagnosis of hepatic steatosis by demonstrating the signal loss, and techniques such as MRI spectroscopy and the newly developed proton density fat fraction can evaluate liver fat quantitatively[8,13]. Focal hepatic steatosis is usually detected on US as a hyperechoic focus and may be mistaken for a metastasis and vice versa[14,15]. The focal fatty infiltrations of the liver are described as geographically bordered lesions without a compression effect, usually localized in the gallbladder fossa, segment 4, and the areas neighboring the falciform ligament and portal vein[14]. Similarly, in this study, 9 of the 10 (90%) lesions defined as focal steatosis were located at segment 4. Seven (70%) lesions had geographical contours, while none had a compression effect. These results show that even though the diagnostic spectrum of hyperechoic focal liver lesions detected on US comprises many pathologies involving malignancies, MRI may be unnecessary in the presence of lesions with typical contour characteristics, which do not possess a hypoechoic halo or have a compression effect on the adjacent structures. Although it is not yet fully understood and moderately differentiated, HCCs may

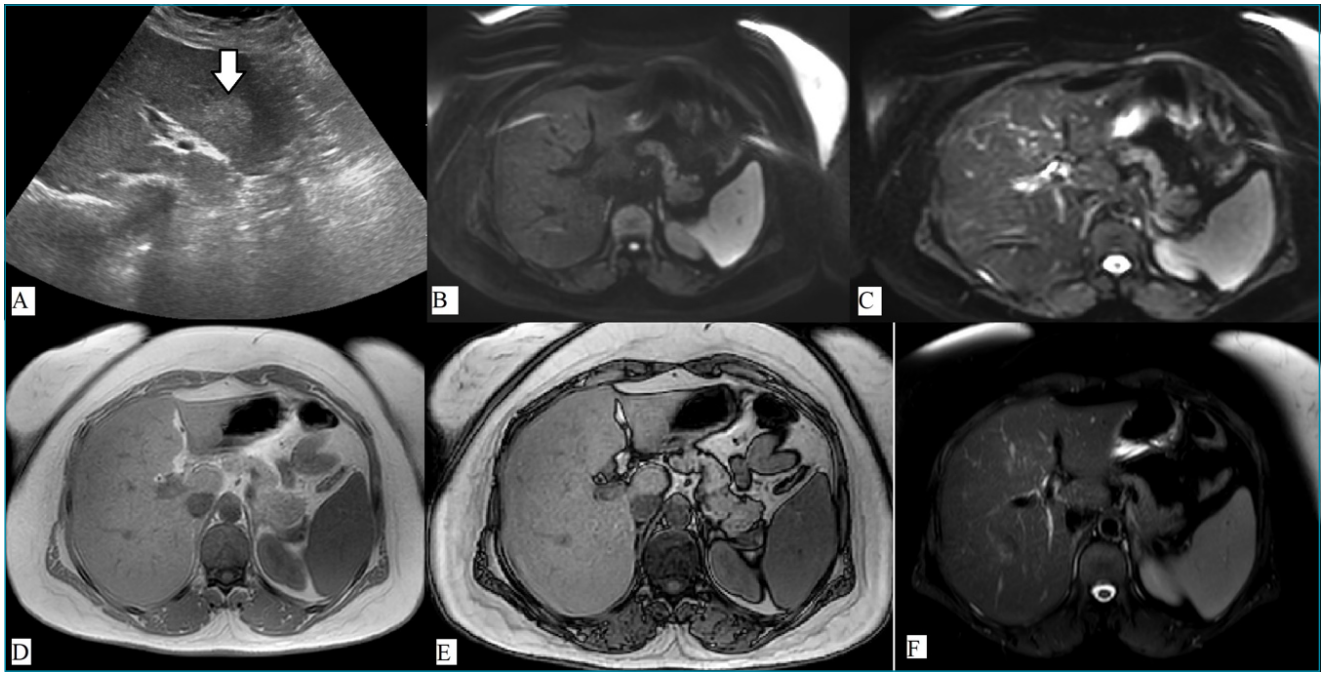

Figure 1. (A) Ultrasonography clearly demonstrates a hyperechoic area in the left lobe of the liver. (B), (C), (D), (E), and (F) No lesion is detected on diffusion weighted imaging, dual echo, and T2-weighted images. Images of a 43-year-old woman.

include fat and appear hyperechoic on US[5]. The literature indicates that small HCC lesions may have a higher chance of appearing hyperechoic on US[16]. In this study, a total of four lesions in two patients had received a diagnosis of HCC. Three of these lesions were 2 cm or less in size, and all comprised focal heterogeneous areas (Figure 2).

The number of studies in the literature concerning focal liver lesions detected incidentally on US is rather limited. In a retrospective study performed by Kaltenbach et al., the incidence of hepatic benign lesions detected by re-scanning the previously performed US examinations was found to be 15.1%. In that study, the most frequent focal pathology was reported to be focal fatty sparing, with an incidence of 6.3%[17]. According to the results of this study, the most frequent hyperechoic lesions were typical hemangiomas (56.9%), followed by the lesions of focal hepatic steatosis (13.9%). We should emphasize that among the diagnoses acquired by MRI were benign pathologies such as hydatid cysts and lipomas, as well as malignant pathologies such as HCC. This is an important finding necessitating a thorough investigation of the history and clinical findings of patients in addition to their US imaging findings (Figure 3).

In this study, area measurements, together with their contour characteristics and segmental localizations, were compared between the US and MRI examinations. A carefully performed US examination can provide very similar results to an MRI examination. In contrast, it is also clear that US is usually sufficient in the follow-up of certain characteristics of previously diagnosed lesions, such as their numbers and lesion area measurements. To the best of our knowledge, in the English language literature, these lesion characteristics have not been previously compared these two imaging modalities. Our findings should be verified by similar further studies performed with larger patient cohorts.

The relatively low number of patients included in this study is a limitation. We think that further studies that will be performed with larger patient groups will disclose the presence of other benign and malignant lesions. Another limitation of this study is that the diagnoses were made based on MRI findings, and biopsy procedures could not be performed due to ethical reasons. The evaluation of follow-up imaging findings may overcome this problem.

CONCLUSIONS

Incidentally detected hyperechoic zones may not necessarily be detected on MRI secondary to mild focal hepatic steatosis or false interpretation of the radiologist. Although hemangioma is the usual suspect in the focal hyperechoic lesions of the liver detected on US, lesions requiring therapy must also be considered in the differential diagnosis. A thorough evaluation of these patients can be undertaken by acquiring detailed clinical data, scrutinizing sonographic and MRI findings, and performing a biopsy procedure if necessary.

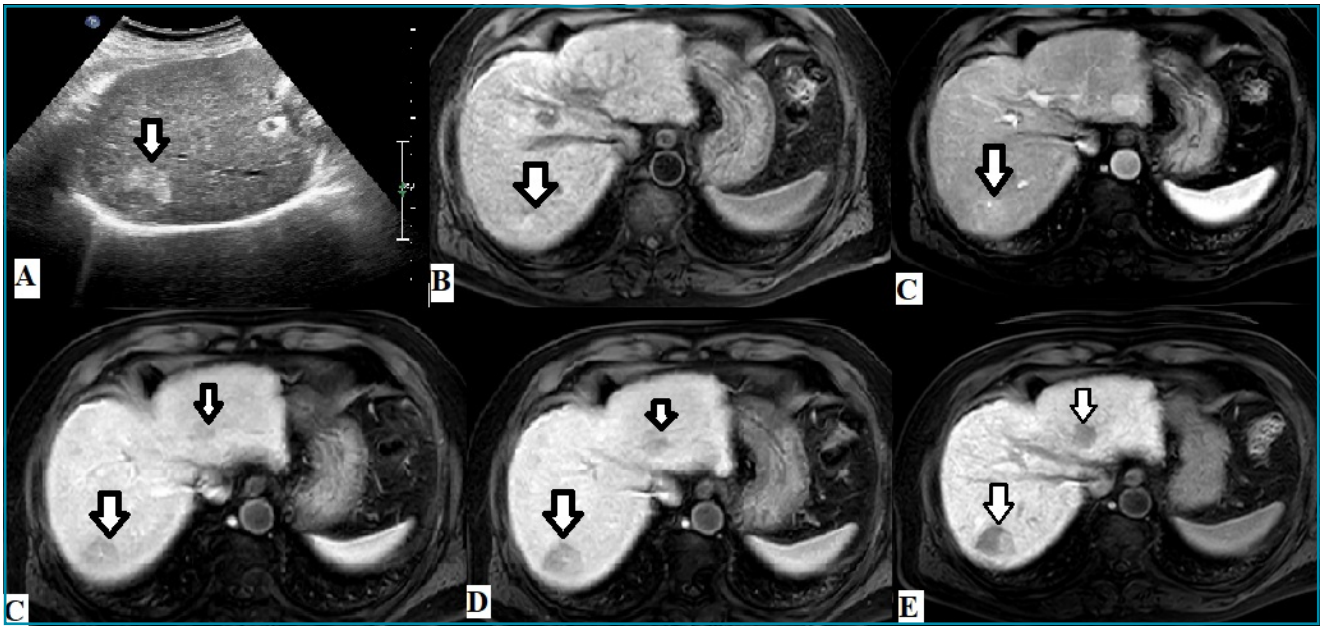

Figure 2. (A) A hyperechoic lesion with hypoechoic areas at segment 7 on ultrasonography (another hyperechoic lesion is not shown). (B) The lesion is slightly hyperintense on the axial T1-weighted image. (C), (D), and (E) Axial T1-weighted image shows arterial hyperenhancement and wash out in portal and venous phases. (E) Hepatobiliary phase lesions are hypointense. Images of a 70-year-old man with hepatocellular carcinoma.

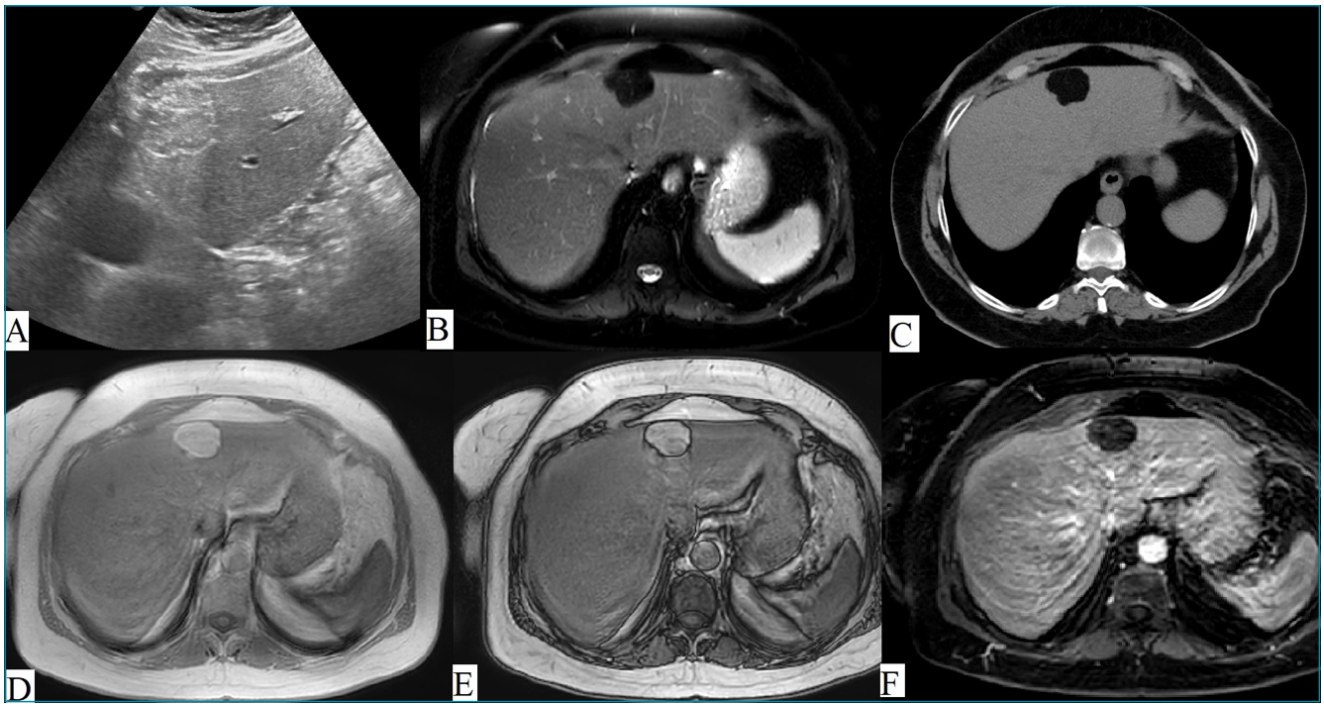

Figure 3. (A) Ultrasonography image reveals a hyperechoic lesion at segment 2 of the liver. (B) Axial fat-saturated T2-weighted image shows a hypointense lesion. (C) In the axial computed tomography image, the lesion is hypodense. (D) and (E) Dual gradient echo in-phase and opposed-phase images demonstrate a hyperintense lesion with a chemical shift artifact at the periphery of the lesion. (F) There is no contrast enhancement on the axial T1-weighted image. Images of a 58-year-old woman with a hepatic lipoma.

AUTHORS' CONTRIBUTIONS

GS: Conceptualization, Data curation, Writing – original draft. **SL:** Conceptualization, Data curation. **OD:** Conceptualization, Data curation. **HA:** Data curation. **OK:** Data curation. **II:** Conceptualization, Formal Analysis. **CY:** Supervision. **BG**: Writing – review & editing.

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ORIGINAL ARTICLE
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Evaluating causative agents, mortality factors, and laboratory data of hospital-acquired pneumonia patients

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SUMMARY

OBJECTIVE: In the recent years, the increase in death rates from nosocomial pneumonia draws attention. The aim of this study was to examine the causative agents and mortality factors of patients with pneumonia who were followed up in the chest diseases intensive care unit.

METHODS: Data of 1070 patients with pneumonia were screened for this study. A total of 160 patients with hospital-acquired pneumonia included in this study. The relationship between factors such as patients' comorbidities, length of stay in the intensive care unit, history of hospitalization or respiratory support therapy, infection markers such as C-reactive protein, white blood cell, nutritional markers such as albumin and protein, renal and liver function tests, culture growing microorganisms, and clinical pulmonary infection scores was evaluated and mortality rates were examined.

RESULTS: Among 1070 patients, the rate of hospital-acquired pneumonia was 14.9%, and the mortality rate of pneumonia was 16.9%. Mortality was significantly increased in patients who stayed in the intensive care unit for more than 10 days, in patients with a clinical pulmonary infection score of ≥6 and with a history of hospitalization in the past one month, and received invasive mechanical ventilation therapy. Mortality increased in patients with hypoalbuminemia, hypoproteinemia, and high C-reactive protein values. The most commonly grown microorganism was *Acinetobacter baumannii,* which was also found significantly in patients who underwent invasive mechanical ventilation.

CONCLUSION: In the clinical approach to hospital-acquired pneumonia, in order to prevent mortalities, it is important to reveal whether the newly emerging symptoms and signs are related to pneumonia, to identify the causative pathogen, and to determine the severity of the disease.

KEYWORDS: Hospital acquired pneumonia. Intensive care unit. Mortality.

INTRODUCTION

Hospital-acquired pneumonia (HAP) is the most common hospital-acquired infection[1]. HAP is defined as pneumonia that usually develops 48 h after hospitalization without incubation period at the time of hospitalization and that occurs within 48 h of discharge from the hospital[2].

The time of onset of pneumonia emerges as an important variable in the prognosis of HAP patients in terms of both epidemiological and problematic microbiological factors. Early-onset HAP that develops within the first 4 days after hospitalization is generally benign and has a good prognosis due to treatable microorganisms. Late-onset HAP is the disease that develops on the fifth and subsequent days, with serious pathogens that show multidrug resistance (MDR), cause treatment problems, and increase treatment cost[3-5]. Microorganisms in the etiology of HAP may vary with the

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underlying disease, presence of risk factors, and the time of occurrence of pneumonia[6].

The aim of this study was to examine the causative agents, mortality factors, the contribution of laboratory data to clinical outcome and mortality of patients, and the contribution of clinical pulmonary infection score (CPIS) to the prognosis of the pneumonia patients who were followed up in the Chest Diseases Intensive Care Unit of Erciyes University Faculty of Medicine.

METHODS

This study was carried out retrospectively in "hospital-developing pneumonia" patients who stayed in the Chest Diseases Intensive Care Unit of Erciyes University Medical Faculty Hospital between June 2006 and June 2011 for more than 48 h, who were known to have no previous pneumonia, and whose secretions increased in the first 48 h of discharge. This study was approved by the Research Ethics Committee of Erciyes University Medical Sciences (resolution number: 2011-361).

The registry archive of the chest diseases intensive care unit (ICU) was scanned for the study. The profiles, file numbers, and clinical and laboratory data of the 1070 patients were obtained. Hospitalization history of these patients before their epicrisis, antibiotics usage before admission to intensive care, comorbidities, course of fever, length of stay in the ICU, CPIS value, mortality, facilitating risk factors, clinical navigational information, etiological microorganisms, empirical antibiotics usage, and antibiotic susceptibilities were recorded. To support the data, the control records of the infection committee were scanned with the permission of the infectious diseases control committee. Patients who were discharged from the ICU in the first 24 h were excluded from the study. Liver and renal function tests, albumin, protein, magnesium (Mg), C-reactive protein (CRP) levels, and complete blood count parameters were recorded. The risk factors for HAP such as the presence of central venous catheter, tracheostomy, reintubation, bronchoscopy, tube thoracoscopy, percutaneous endoscopic gastrostomy (PEG), urethral catheter, nasogastric tube, history of total parenteral nutrition (TPN), cytostatic agents, transfusion, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, underlying malignancy, central nervous system pathology, bronchiectasis, hemodialysis admission, metabolic acidosis, and length of stay in ICU were recorded and factors affecting mortality rates were examined.

Statistical analysis

Data analysis was done using Statistical Package for Social Science version 11.5 package program. Shapiro–Wilk test was used for the normally distributed continuous data. Descriptive statistics were presented as number of cases, median, and lowest and highest values for continuous variables and (%) for categorical variables. Categorical variables were evaluated with Pearson's chi-square or Fisher's exact test. Continuous variables were analyzed by univariate logistic regression analysis. As a result of univariate statistical analyses, variables determined as $p<0.25$ were accepted as candidate risk factors in distinguishing survivors and exitus groups. Among all possible risk factors, the most determining factors were investigated by multivariate logistic regression analysis. Odds ratio and 95% confidence interval for each variable were calculated. For $p<0.05$, all the results were considered statistically significant.

RESULTS

The names and protocol numbers of 1070 patients were obtained from the records of the Intensive Care Unit of Erciyes University Faculty of Medicine. Out of 1070, 226 patients were excluded from this study because they were discharged from the ICU or died in the first 24 h. Of the 844 patients whose records could be accessed, 160 (18.9%) patients were identified with microorganisms growing 48 h after they were admitted to the ICU and/or in the first 48 h of discharge. The clinical and demographic data of these 160 patients who met all the study criteria were examined. CRP, white blood cell (WBC), blood urine nitrogen (BUN), creatinine, aspartate transaminase (AST), analine transaminase (ALT), and Mg values at the date of reproduction of the microorganisms were recorded from the database.

Among 160 patients, 97 (60.6%) were males and 63 (39.4%) were females. The mortality rate was 61.9% (n=99). Of 99 patients, 60 (60.6%) were males and 39 (39.4%) were females (p=0.959). A total of 115 (71.9%) patients who developed HAP were ≥65 years of age. Over 75.8% (n=75) of the patients who died during the hospitalization were ≥65 years. The difference between the rates of mortality in ≥65 years was not statistically different (p=0.353).

Considering underlying diseases, 53.1% (n=85) of the patients had chronic obstructive pulmonary disease, 22.5% (n=36) had hypertension, 16.9% (n=27) had diabetes mellitus, and 4.4% (n=7) had bronchiectasis. Central nervous system pathology was present in 11 (6.9%) patients. Mortality rates were examined and we found that 27.7% (n=26) patients had hypertension, 53.7% (n=51) had chronic obstructive pulmonary disease, 9.7% (n=9) had underlying malignancy, 17.9% (n=17) had hemodialysis admission, 17.7% (n=17) had diabetes mellitus, 7.3% (n=7) had central nervous system pathology, and 1.0% (n=1) had bronchiectasis. Bronchiectasis and hemodialysis admission were significantly associated with mortality (p=0.013 and p=0.022, respectively) (Table 1).

Notably, 131 (81.9%) patients were treated in invasive mechanical ventilation (IMV) and 62 (38.8%) patients were

treated in non-invasive mechanical ventilation (NIMV). The number of patients receiving only oxygen therapy was 7 (4.4%). In addition, 90.9% of the deceased patients were treated in IMV (n=90), and three patients received oxygen therapy during their hospitalization. There was a statistically significant relationship between IMV and mortality (p<0.001) (Table 1).

Effects of laboratory parameters on mortality rates are given in Table 2. The difference between WBC levels in two groups was not statistically significant (p=0.254). However, the difference between mean CRP levels of two groups and its relation with mortality was statistically significant (p=0.003). Although the mean protein values were 5.2 (2.3–7.7), there was a weak statistically significant correlation with mortality (p=0.049). The mean albumin value of the patients in exitus group was 2.3 (1.0–3.8), and its relationship with mortality was also considered statistically significant (p=0.02). No significant correlations were found between other laboratory values and mortality.

Among 86 patients whose CPIS could be calculated, the mean CPIS value of the surviving patients was 7 (3–10), while the mean CPIS value of the deceased patients was eight (4–10). The correlation between CPIS level and mortality was statistically significant (p=0.013). Of 86 patients whose CPIS value was calculated, 59 died and 57 had a CPIS value of ≥6 (96.6%). This result showed that there was a statistically significant relationship between mortality and CPIS ≥6 (p=0.004).

While a single microorganism was found to be responsible in 131 cultures, it was recorded as polymicrobial in 29 cultures. Samples were taken from endotracheal aspirate (ETA) (n=108), bronchoalveolar lavage (BAL) fluid (n=31), and sputum (n=21). Pneumonia agent was also isolated in blood culture in 16 out of 160 patients who developed HAP. These isolates were *Acinetobacter baumannii* (n=9), *Staphylococcus aureus* (n=3), *Escherichia coli* (n=2), *Klebsiella pneumoniae* (n=1), and *Enterococcus faecium* (n=1). The most commonly isolated microorganisms were, in order of frequency, *A. baumannii* (n=71),

Table 1. Effects of underlying diseases and respiratory support on mortality rates.

	Alive	Exitus	p-value	OR (95%CI)
Hypertension, n (%)				
(+)	48 (82.8)	68 (72.3)	0.142	1.835 (0.810–4.157)
(–)	10 (17.2)	26 (27.7)		
Chronic obstructive pulmonary disease, n (%)				
(+)	25 (42.4)	44 (46.3)	0.632	0.852 (0.443–1.641)
(–)	34 (57.6)	51 (53.7)		
Malignancy, n (%)				
(+)	55 (94.8)	84 (90.3)	0.373	1.964 (0.509–7.578)
(–)	3 (5.2)	9 (9.7)		
Diabetes mellitus, n (%)				
(+)	49 (83.1)	79 (82.3)	0.904	1.054 (0.447–2.488)
(–)	10 (16.9)	17 (17.7)		
Central nervous system pathology, n (%)				
(+)	55 (93.2)	89 (92.7)	1.000	1.081 (0.303–3.865)
(–)	4 (6.8)	7 (7.3)		
Bronchiectasis, n (%)				
(+)	53 (89.8)	95 (99.0)	0.013	0.093 (0.011–0.793)
(–)	6 (10.2)	1 (1.0)		
Hemodialysis admission, n (%)				
(+)	56 (94.9)	78 (82.1)	0.022	4.068 (1.137–14.552)
(–)	3 (5.1)	17 (17.9)		
Invasive mechanical ventilation, n (%)	40 (67.8)	90 (90.9)	<0.001	4.750 (1.978–11.408)
Oxygen therapy, n (%)	4 (6.8)	3 (3.0)	0.426	0.430 (0.093–1.991)

OR: odds ratio; CI: confidence interval.

E. coli (n=28) (extended spectrum beta-lactamase [ESBL] production; n=18), *Pseudomonas* spp. (n=25), *Klebsiella* spp. (n=17), *S. aureus* (n=17) (methicillin-susceptible [MSSA] n=6 and methicillin resistant [MRSA] n=11), Gram (–) bacilli (n=12), *Proteus* spp. (n=6), *Streptococcus* spp. (n=4), *Enterobacteriaceae* spp. (n=3), and others (*Stenotrophomonas maltophilia*, *Serratia* marcescens, *Citrobacter* koseri, and fungal agents) (n=15). *Proteus* spp. was found as a candidate risk factor for mortality (p=0.085). However, there were no significant relationships between the other microorganisms grown in the cultures of the patients and their mortality rates (Table 3).

DISCUSSION

The defense mechanisms of the patients hospitalized in the ICU are generally impaired due to existing or accompanying diseases. Immune paralysis occurs due to the release of anti-inflammatory mediators and is associated with an increased risk of infectious complications. Immunosuppressive therapies, which are frequently applied to intensive care patients, contribute to these complications and convey patients to the high-risk group for nosocomial infection[7]. Endotracheal intubation reduces local defense mechanisms and paves the way for respiratory tract infections. In the first 24 h after admission to the ICU, the mouth is colonized by pathogenic bacteria[8]. In the first 48–72 h, 50% of the patients, and at the end of the first week almost all patients, are colonized by the factors that make up the flora of the unit. Colonization rate in critically ill patients reaches up to higher rates[9]. In nosocomial pneumonia, there is usually microaspiration or macroaspiration of these colonized microorganisms.

The main factors in early-onset HAP are not different from community-acquired pneumonia, and the most common are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and MSSA[1]. In late-onset pneumonia, *P. aeruginosa*, *Acinetobacter* spp.,

Table 2. Effects of laboratory parameters and CPIS on mortality rates.

	Alive Mean (95%CI)	Exitus Mean (95%CI)	p-value
Albumin (g/dL)	2.5 (1.2–3.8)	2.3 (1.0–3.8)	0.020
Total Protein (g/dL)	5.3 (3.9–7.6)	5.2 (2.3–7.7)	0.049
Mg (mg/dL)	0.8 (0.6–1.4)	0.9 (0.3–1.7)	0.611
WBC ($\times 10^3/mm^3$)	14 (5–36)	15 (2–58)	0.254
AST (IU/L)	32 (10–561)	35 (5–2040)	0.370
ALT (IU/L)	31.5 (5–937)	26 (0.8–1614)	0.863
BUN (mg/dL)	30 (9–86)	32 (9–131)	0.096
Creatinine (mg/dL)	1 (0.4–2.8)	1.1 (0.3–38)	0.144
CRP (mg/L)	62 (1.3–339)	119 (3–638)	0.003
CPIS	7 (3–10)	8 (4–10)	0.013

WBC: White blood cell; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urine nitrogen; CRP: C-reactive protein; CI: confidence interval; CPIS: clinical pulmonary infection scores.

Table 3. Effects of microorganisms on patients' mortality rates.

	Alive (n=59)	Exitus (n=99)	p-value
Acinetobacter spp. (%)	23 (39.0)	46 (46.5)	0.359
E. coli (%)	9 (15.3)	19 (19.2)	0.531
Enterobacteriaceae spp. (%)	1 (1.7)	2 (2.0)	1.000
Gram (–) bacilli (%)	5 (8.5)	7 (7.1)	0.763
Klebsiella spp. (%)	6 (10.2)	11 (11.1)	0.853
Proteus spp. (%)	–	6 (6.1)	0.085
Pseudomonas spp. (%)	10 (16.9)	15 (15.2)	0.765
Staphylococcus aureus (%)	6 (10.2)	10 (10.1)	0.989
Streptococcus spp. (%)	2 (3.4)	2 (2.0)	0.630
Others (%)	8 (13.6)	7 (7.1)	0.178

Enterobacter spp., *Klebsiella* spp., Gram-negative agents, and *S. aureus* can be seen as a factor in 20–30% of cases[1,10]. The prevalence of *Legionella* species is reported to be 10–20% and its frequency increases in cases of corticosteroid use, immunosuppression, and previous antibiotic use[11]. *Legionella pneumophila* should be considered among the causative agents of HAP in patients hospitalized in the ICU[11].

The early or late stage of HAP, the underlying risk factors, and the severity of pneumonia shape the empirical treatment[12]. Appropriateness in empirical treatment is possible with the correct estimation of the origin organisms and requires rational antibiotic use skills that require clinical and microbiological knowledge, including local resistance data from the ICU.

Mortality rates of lower respiratory tract infections in hospitalized patients are high. Mortality is much higher in pneumonia developing especially in ventilator-dependent patients[13]. In our study considering underlying diseases, bronchiectasis and hemodialysis admission were significantly associated with mortality (p=0.013 and p=0.022, respectively) in HAP patients. There was also a statistically significant relationship between IMV and mortality (p<0.001).

It is believed that the most important cause of HAP is the colonization of the gastrointestinal tract and oropharynx by pathogenic microorganisms[14,15]. Pneumonia occurs with aspiration of these pathogens and failure of the host defense[14]. Microaspiration of oropharyngeal secretions may occur during sleep in normal individuals. However, these aspirates are small and originated from nonpathogenic flora. However, in hospitalized patients, oropharyngeal colonization occurs with pathogenic aerobic Gram-negative bacilli. Colonization is facilitated by coma, hypotension, acidosis, azotemia, alcoholism, diabetes mellitus, leukocytosis, leukopenia, pulmonary disease, nasogastric and endotracheal intubation, and antibiotic utilization. In these patients, the aspirated volume and frequency of aspiration also increase for various reasons such as altered consciousness, difficulty in swallowing, decreased gurgling reflex, delayed gastric emptying, and slowing of gastrointestinal motility[16]. Normally, gastric acidity prevents pathogenic microorganisms. However, in cases of advanced age, achlorhydria, ileus, upper gastrointestinal system diseases, use of antacids or H2 receptor antagonists, proton-pump inhibitors, and enteral nutrition, the gastric pH rises above 4 and gastric acid protection disappears. In the HAP diagnosis and treatment management guidelines published by the American Thoracic Society in 2005, common pathogens in HAP cases are Gram-negative bacilli such as *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *Acinetobacter* spp. Gram-positive cocci, especially MRSA and anaerobes. In the 2008 HAP Guideline of the Turkish Thoracic Society, common pathogens in late-onset HAP were indicated as *P. aeruginosa*, *Acinetobacter* spp., *Enterobacter* spp., and *Klebsiella* spp. However, Hu et al. reported that agent spectrum and antibiotic susceptibility differ in late-onset Group 2 and Group 3 HAP cases[17]. The most common isolated microorganisms in our study were, in order of frequency, *A. baumannii* (n=71) and *E. coli* (n=28) (ESBL production; n=18). *Proteus* spp. (n=6) was found as a candidate risk factor for mortality (p=0.085). However, there were no significant relationships between the other microorganisms grown in the cultures of the patients and their mortality rates (Table 3).

Laboratory tests have been performed to complete the diagnosis and to guide clinicians about the prognosis of HAP. Zheng et al. showed that the neutrophil/lymphocyte count ratio, procalcitonin, and CRP levels were markedly different between the non-infection and HAP groups[18]. In our study, the effects of laboratory parameters on mortality rates were also examined and the difference between mean CRP and albumin levels of groups and their relations with mortality were found statistically significant (p=0.003 and p=0.02, respectively). We also examined the relation between CPIS and mortality and our study showed that there was a statistically significant relationship between mortality and CPIS ≥6 (p=0.004).

Our study had some limitations. First, this was a retrospective study evaluating causative agents, mortality factors, laboratory data to clinical outcome and mortality of patients, and the contribution of CPIS to prognosis of patients with HAP. Therefore, our study could be improved by performing further prospective studies. Second, we examined a limited number of laboratory parameters and inflammation markers. Further prospective studies are required to clarify the effects of laboratory parameters and inflammation markers on prognosis and mortality rates of patients with HAP.

CONCLUSIONS

The clinical diagnosis of HAP is difficult. Infectious and non-infectious pathologies should be considered in the differential diagnosis. Difficulty in diagnosis causes unnecessary antibiotic utilization, and as a result, there is an increase in risk of antibiotic-resistant bacterial infection, toxicity, and treatment cost. In addition, mortality rates of these patients are high. Hence, combining clinical, laboratory, and CPIS data and isolating the related pathogens earlier with appropriate antibiotics therapy may help clinicians to predict the prognosis of patients with HAP.

AUTHORS' CONTRIBUTION

EO: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **SG:** Conceptualization, Data curation, Formal analysis, Resources, Software, Writing – review & editing. **NC:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Long-term follow-up results of unfractionated heparin infusion treatment for submassive pulmonary thromboembolism

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SUMMARY

OBJECTIVE: Treatment options for submassive pulmonary thromboembolism cases vary depending on the patient's hemodynamic stability, comorbidities, and bleeding risk. The long-term effect of unfractionated heparin treatment on pulmonary hypertension and mortality is unclear. The aim of this study was to investigate the long-term effect of unfractionated heparin treatment on pulmonary thromboembolism.

METHODS: This is a cross-sectional study with 22 patients who were diagnosed with submassive pulmonary thromboembolism and followed up at the outpatient clinic between 2016 and 2020 and received unfractionated heparin treatment.

RESULTS: Mean pulmonary artery pressure was 53±13.6 mmHg during hospital admission and 42.7±13.4 mmHg at hospital discharge. There was a statistically significant decrease in D-dimer and pulmonary artery pressure levels before and after treatment (p=0.001). At the end of one year, pulmonary artery pressure was considered high in three patients of this study.

CONCLUSION: Our study suggests that unfractionated heparin is safe in the treatment of submassive pulmonary thromboembolism in terms of bleeding risk and reduces pulmonary artery pressure.

KEYWORDS: Pulmonary embolism. Unfractionated heparin. Long-term effect. Pulmonary hypertension.

INTRODUCTION

Pulmonary thromboembolism (PTE) presents with different clinical characteristics, ranging from asymptomatic cases to those who die within hours due to hemodynamic instability. Due to these clinical differences, treatment approaches also vary. "Risk assessment" is the most critical step in determining the appropriate treatment approach for acute PTE cases. "Risk," as defined herein, is the risk of death associated with the acute PTE. Therefore, distinguishing the patient diagnosed with acute PTE as high risk (massive), intermediate risk (submassive), or low risk (nonmassive) in terms of early mortality can help determine the treatment options and prognosis[1]. Low-molecular-weight heparin (LMWH) and oral anticoagulants are often preferred for the treatment of nonmassive PTE and thrombolytics for massive PTE[2]. Treatment options for submassive PTE cases vary depending on the patient's hemodynamic stability, comorbidities, and bleeding risk[3]. Systemic anticoagulation, LMWH, oral anticoagulants, catheter-directed thrombolysis, half-dose thrombolysis (50 mg tPA), and inferior vena cava (IVC) filters are the treatment options for submassive PTE[4,5]. Unfractionated heparin (UFH) treatment was commonly used in the past for submassive PTE but is less preferred nowadays due to newer treatment options that are easier to follow. Studies investigating the effect of UFH infusion therapy on pulmonary hypertension (PHT) in the long term are insufficient.

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In this article, we examined the demographic characteristics, symptoms, comorbidities, and risk factors of patients with submassive PTE treated with UFH infusion; the effect of heparin on platelet count and blood count in the early (3–5 days) and late (15 days) periods; and the developmental status of chronic thromboembolic PHT at the end of one year.

METHODS

In our study, data from 22 patients who received UFH infusion therapy after admission to the intensive care unit (ICU) with a diagnosis of submassive PTE and were subsequently followed up in the outpatient clinic for one year were retrospectively analyzed between 2016 and 2020 according to the 2015 Turkish Thoracic Society PTE diagnosis and treatment consensus report.

Study population

The diagnosis of PTE was made in the emergency department using contrast-enhanced dynamic chest computed tomography (image). Patients with findings consistent with submassive PTE on echocardiography (ECHO) were included in this study. In addition, patients with findings of right ventricular dilatation, paradoxical motion and deviation of the septal wall to the left, moderate or severe hypokinesis suggestive of right ventricular dysfunction, mobile thrombus in the right atrium, PHT, and patent foramen ovale at ECHO, despite normal systemic blood pressure, were considered submassive PTE. Patients' symptoms on admission, concomitant diseases and risk factors, hemoglobin and platelet counts, troponin and D-dimer levels, arterial blood gas values, and ECHO findings on admission to the ICU and on discharge from the hospital were obtained from hospital records. Additionally, follow-up results of ECHO at months one, three, and six and at the end of the first year, as well as D-dimer levels at the end of treatment, were obtained from outpatient records. Subsequently, all collected data were statistically analyzed.

Treatment protocol

All patients were followed up in the ICU after ECHO was performed in the emergency department. After observing the basal activated partial thromboplastin time (aPTT) values of the patients, a bolus of 80 IU/kg intravenous (i.v.) followed by a heparin infusion of 18 IU/kg/h was started. In the first 24 h, treatment was supplemented with warfarin when the aPTT value reached 45–70. The aPTT level was measured every 6 h for the first 24 h and daily after reaching the desired level. If internalized normal ratio (INR) values of 2–3 were detected within the 24-h interval, heparin was discontinued and treatment with warfarin was continued. Thus, all patients received warfarin therapy for at least six months.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 15.0. A homogeneity test was performed on numeric variables. Independent sample *t*-tests were used for numerical parameters. Paired sample *t*-test was used to test whether there was a difference in the mean of numerical variables at the beginning (baseline) and end of treatment. Pearson's correlation analysis was performed for distributed homogeneous variables. Nonhomogeneous variables were analyzed using nonparametric tests. A $p<0.05$ was considered significant.

RESULTS

Of the 22 patients followed up and treated with a diagnosis of submassive PTE, 11 were females and 11 were males. The mean age of the cases was 53.5±20 years. The most common symptoms on admission were shortness of breath (n=22), central chest and flank pain (n=18), palpitations (n=16), and cough (n=15), while the most common risk factors were immobilization (n=12), deep vein thrombosis (n=10), and orthopedic surgery (n=6) (Table 1).

Blood gas values of patients on admission were as follows: pH: 7.46±0.05, PO_2: 66.1±14.1 mmHg, PCO_2: 32.4±5.2 mmHg, HCO_3: 22.1±3.1 mEq/L, saturation O_2: 92±4.7%. Hypoxemia (PO_2<80 mmHg) was observed in 18 patients, and hypocapnia (PCO_2<35 mmHg) was observed in 15 patients.. D-Dimer levels were 3.67±2 ng/mL before treatment and 0.49±0.59 ng/mL at the end of treatment. D-Dimer levels remained high (>0.5 ng/mL) in two patients at the end of treatment. Of the patients whose D-dimer levels remained high, one had prior cerebrovascular disease and coronavirus 2019 disease, and one had breast carcinoma.

The mean troponin level on admission was 42.3±50.3 ng/mL. Troponin levels were above normal laboratory values in 12 patients (troponin 0–14 ng/mL).

Mean hemoglobin on hospital admission was 12.2±2.1 and 11.2±1.9 g/dL on day three, 11.7±1.8 g/dL on day five, and 11.9±1.6 g/dL on day 15. The mean platelet count was 291,500±95,700/mL on admission, 295,000±159,100/mL on day 3, 307,300±158,000/mL on day 5, and 341,800±159,000/mL on day 15. No patient experienced a decrease in hemoglobin level or platelet count during the early (on days 3–5) and late (on day 15) phases with heparin.

Mean pulmonary artery pressure (PAP) was 53±13.6 mmHg on ECHO in the emergency department during hospital admission. PAP was found to be 42.7±13.4 mmHg at hospital discharge (ECHO) (Table 2). There was a statistically significant decrease in D-dimer and PAP levels before and after treatment. Notably, 19 patients with D-dimer levels below 0.5 ng/mL had PAP levels of 20 mmHg and below at the end of treatment. At the end of 1 year, PAP was considered high in three patients. It was determined that three patients underwent V/P scintigraphy and were evaluated in favor of chronic thromboembolic PHT, and one patient underwent endarterectomy (Figure 1).

Table 1. Demographics, symptoms, comorbidities, and risk factors.

	n
Age (mean±SD)	53.5±20.4
Male/Female, n	11/11
Symptoms	n (%)
Shortness of breath	22 (100)
Central chest and flank pain	18 (81.8)
Palpitation	16 (72.7)
Cough	15 (68.2)
Pain, redness, swelling in the leg	13 (59.1)
Wheezing	9 (40.9)
Producing sputum	2 (9.1)
Hemoptysis	1 (4.5)
Concomitant diseases and risk factors	n (%)
Immobilization	12 (54.5)
Hypertension	12 (54.5)
Deep vein thrombosis	10 (45.5)
Orthopedic surgery	6 (27.3)
Heart failure	6 (27)
Diabetes	5 (22.7)
Obesity	5 (22.7)
Cesarean section	4 (18.2)
Neurosurgery operation	1 (4.5)
Lymphoma	1 (4.5)
COVID-19	1 (4.5)
Breast carcinoma	1 (4.5)
Cerebrovascular disease	1 (4.5)

SD: standard deviation.

Table 2. Mean value of pulmonary artery pressure at 1-year follow-up of patients.

ECHO	PAP (mean±SD)
At hospitalization	53.0±13.6
At discharge from hospital	42.7±13.4
At month 1	37.6±12.6
At month 3	30.2±13.1
At month 6	25.4±10.1
At the end of 1 year	23.8±9.7

ECHO: Echocardiography; PAP: pulmonary artery pressure; SD: standard deviation.

DISCUSSION

When PTE is diagnosed, anticoagulant therapy should be started as soon as possible unless contraindications exist. In our study, the long-term outcomes of 22 patients diagnosed with submassive PTE and treated with UFH infusion were evaluated; accordingly, their PAP and D-dimer levels decreased significantly during follow-up, and PHT developed in three patients due to chronic PTE (CPTE).

Treatment of submassive PTE may be determined depending on the patient's clinical condition, drug contraindications, comorbidities, and hemodynamic findings. Although the role of systemic thrombolytic therapy is controversial, patients with clinical deterioration of submassive PTE are potential candidates for thrombolytic therapy[6]. A double-blind, randomized trial showed lower mortality with alteplase than with heparin alone in recurrent PTE, without the added risk of bleeding[7]. Another study comparing tenecteplase and heparin and examining 1,006 patients with submassive PTE reported less hemodynamic instability and mortality in the tenecteplase group[8]. The study by Rehman et al., which examined 86 patients with submassive pulmonary embolism, compared patients who received a

Figure 1. Endarterectomy tissues removed from pulmonary artery branches.

thrombolytic followed by a heparin infusion with those who received a heparin infusion alone and concluded that the PAP scores of patients who received an early thrombolytic followed by a heparin infusion decreased significantly[9]. Three-year follow-up data from the PEITHO trial showed no difference in long-term mortality or incidence of chronic thromboembolic PHT between the thrombolytic and heparin infusion groups[8].

The incidence of chronic thromboembolic PHT is 0.57% in the general population and 1.5% in patients with idiopathic PTE[1]. Factors predisposing to the development of PHT include recurrent venous thromboembolism and PTE of unknown cause. In our one-year follow-up, no recurrent PTE was detected. Thus, there was no case in which we could not detect the underlying risk factor. Nevertheless, 13.6% of chronic PHT were detected. PAP regressed significantly at one-year follow-up, and although the mean PAP of patients fell below 25 mmHg, this suggests that treatment with UFH does not prevent PHT development due to CPTE in submassive PTE. The main limitation of our study is the small number of patients and its descriptive study design. Studies on which treatment option prevents the development of CPTE in submassive PTE can be investigated with further case–control studies using larger patient collectives. Our descriptive study hypothesizes that UFH does not prevent the development of CPTE.

In a meta-analysis of 1,775 patients by Chatterjee et al., thrombolytic therapy was shown to be beneficial in reducing mortality, although it increased the risk of major bleeding (9.24%) and intracranial hemorrhage (ICH) (1.46%)[4]. Similarly, another meta-analysis by Riera-Mestre et al., which reviewed the outcomes of 1,833 patients, found that thrombolytic therapy reduced mortality despite the increased risk of major bleeding (5.9%) and ICH (1.74%)[5]. Although studies on the use of thrombolytics in the treatment of submassive PTE have been accelerated, there are few studies investigating the use of UFH, which reduces the risk of bleeding and has been used safely for many years, in submassive PTE and its long-term outcomes. In our study, none of the patients developed major bleeding or ICH.

Heparin-induced thrombocytopenia is a complication of PTE[10]. In our study, thrombocytopenia was not found in any of the cases. However, when comparing the initial PAP and PAP at the end of one year, we determined that the values decreased significantly in our cases. PAP was 20 mmHg or less in all, except in three patients. Although one case underwent endarterectomy for CPTE-related PHT, we did not detect any losses in our short- and long-term follow-up.

Post-PTE syndrome[11], which is defined in the literature as a long-term complication that causes functional losses after an acute PTE episode, including a decrease in the patient's quality of life during long-term follow-up, was not studied in our patients. This is another limitation of our study.

CONCLUSIONS

Our study suggests that UFH is safe in the treatment of submassive PTE in terms of bleeding risk and reduces PAP, but its impact on PHT development due to CPTE during long-term follow-up needs to be investigated in further case–control studies with larger patient populations.

AUTHORS' CONTRIBUTIONS

LÖ: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **BÖ:** Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. **BA:** Formal Analysis.

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REVIEW ARTICLE
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Use of radiofrequency for the treatment of urinary incontinence in women: a systematic review

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INTRODUCTION

Urinary incontinence (UI) is defined as involuntary loss of urine[1], affecting 25–45% of women, depending on the population[2]. Its classification is based on the pathophysiology and clinical condition of the patient, with the most common being urgency (UUI), stress (SUI), and mixed (IUM).

The SUI is characterized by the involuntary loss of urine due to a cough or physical exertion[3]. The etiology is multifactorial, which may be related to inadequate support and/or weakening of the pelvic floor muscles (PFM), deficiency in the closing mechanism of the urethral meatus, and reduction of collagen synthesis in the pelvic structures[4,5]. The dysfunction has social and psychological impacts, especially on the well-being of the individual. The main risk factors are age, obesity, menopause, pregnancy, parity, and type of delivery[2].

The treatment varies and depends on the classification of incontinence, the patients' perception of symptoms, and their daily life habits[6]. According to the literature[7,8], surgical techniques, drug therapy, and conservative treatments, such as PFM exercises and electrical stimulation, have been shown to improve UI symptoms. Currently, the search for alternatives and safe treatment methods with high cure rates are the targets of research to avoid surgical interventions. Radiofrequency (RF) therapy has been studied as a possible therapeutic modality for SUI, in addition to its increasing clinical use as a non-surgical and non-pharmacological treatment[9].

RF is a minimally invasive procedure involving the application of alternating current that creates electrical fields and generates heat by conversion only in the treatment area. Its frequency can vary from 30 kHz to 300 MHz, and the energy emission mode depends on the number of electrodes, which can be monopolar, bipolar, tripolar, or multipolar[10]. RF can be used to treat SUI and MUI as it promotes submucosal collagen denaturation in the bladder neck and throughout the lower urinary tract, causing tissue retraction in these structures. Therefore, urinary symptoms are expected to be minimized as they are related to the pathophysiology of these types of incontinence[8].

The search for treatment options that are quick to apply, less invasive, safe, and with fewer adverse effects has been growing rapidly, and this therapeutic modality is a good treatment option. Thus, this review highlights the effectiveness and complications of RF therapy for the treatment of UI in women.

METHODS

This systematic review was conducted from June 2020 to May 2021 by searching articles on the Web of Science, Lilacs, Scielo, PubMed, Cochrane, Pedro, Embase, Science Direct, CINAHL, and Scopus platforms. The following descriptors were used by combination: ["Woman" OR "Women"] AND ["Urinary Incontinence" OR "Urge urinary incontinence" OR "Stress Urinary incontinence"] AND ["Radiofrequency" OR "Radio Waves" OR "Pulsed Radiofrequency Treatment" OR "Radiofrequency Ablation" OR "Radiofrequency Therapy"].

The study population was composed of women with UI using any type of RF as an intervention, and there may be

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divergences between the studies related to cost and time of application. Regarding the comparison of treatments, there were no limitations, and the various treatments included no treatment, placebo, vaginal estrogen, or pelvic floor muscle training (TMAP).

The main outcome was the volume of urinary loss measured using the Pad test. Secondary outcomes were urinary loss measured by voiding diary and impact of UI on quality of life, which were assessed using validated International Continence Society questionnaires. Non-urinary variables, such as alterations related to the vaginal epithelium, vaginal dryness, and sexual function were also considered as secondary outcomes. The systematic review was conducted by two authors to select and evaluate the studies and by another to analyze the differences. The kappa test was performed to analyze the inter-rater agreement.

The following were included: clinical trials regardless of randomization and blinding and prospective studies conducted between 2016 and 2021, without language limitations. The exclusion criteria were as follows: studies with less than 3 months of intervention/follow-up; gray literature; animal and cytological studies; articles whose main objective was to evaluate the use of other types of energy for the treatment of UI; and articles aimed at rejuvenating vaginal, fecal incontinence, vaginal atrophy, and other urinary tract diseases.

The PARSIFAL platform was used to verify duplicate studies and the Mixed Methods Appraisal Tool (MMAT) in the 2011 version to assess the methodological quality of the articles.

RESULTS

A total of 279 articles were identified, of which 70 were duplicates and 181 were excluded after consideration by title and abstract. Six studies (n=247 women) were selected for final analysis (Figure 1). The inter-rater kappa test showed an agreement of 76% (κ=0.76; p<0.001).

Radiofrequency procedure

The studies diverged regarding the devices, parameters, temperature, form of application, and number of sessions. It was observed that the monopolar RF[11,12] and non-ablative[13,14] RF were the most used. Two studies[11,12] used the Viveve system protocol (220 pulses of 90 J/cm² in the vaginal introitus). An article[13] used Spectra G2 Tonederm®, which applies a high frequency of 0.5 MHz. In the study using the Votiva device[15], the parameters were adjusted according to the conditions of the volunteers. An article[14] used the ThermiVa device without specifying the parameters. Another[16] configured the device at 45 W and 4 MHz to supply power to 64 microneedles, 0.2 mm in length, allocated in an area of 8 mm × 8 mm. Of the studies that specified the temperature, it ranged between 39–45°C[13-15].

Two studies[11,12] divided the treatment area into quadrants of the vaginal opening, with each quadrant having five consecutive pulse passages. One study applied RF to the external urethral meatus[13], two[14,15] applied in the vaginal canal and labia majora, and one[17] in the urethral meatus and vaginal wall.

Regarding treatment sessions, two studies[11,12] used one session for group I and two for group II, with an interval of 6 weeks; one study[13] described a weekly application for 5 weeks; one study[15] did not specify; and two others[14,16] performed treatment every 30 days for three months. All studies performed follow-ups, ranging from 1 to 12 months[11-16]. These and other detailed information from the articles are shown in Table 1.

Primary outcomes

The volume of urinary loss was analyzed using the Pad test as the primary outcome[11-13,16]. Allan et al.[11] showed in their 6-month study that the leakage volumes of the absorbent weight were similar between the randomized groups at the final assessment. The authors reported that 69% of the overall sample achieved a >50% improvement in pad weight. In the second study[12], with a 12-month follow-up, there was a difference between the randomized groups in relation to the weight loss of the pad, which was >50%. The group that received the two interventions showed a 54% improvement. In the group that received only one intervention, the evolution rate was 50%.

The study by Lordelo et al.[13] evaluated the individual evolution of each patient with a 1-h Pad test for three months. It was observed that 70% of the patients had a reduction in UI, 20% did not complain of additional loss, and 30% showed worsening of the additional loss.

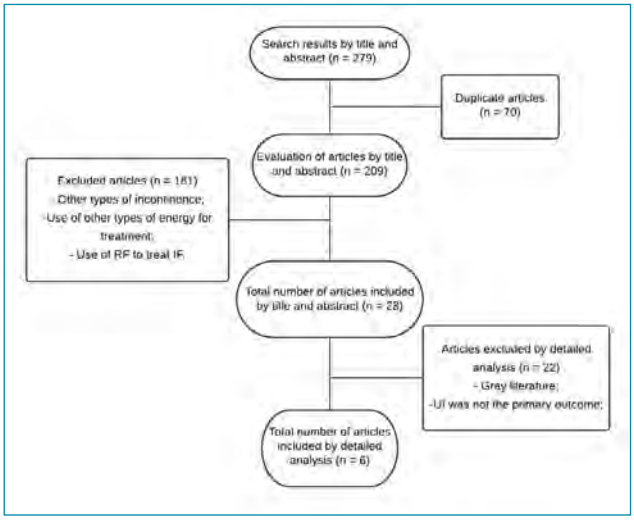

Figure 1. Flowchart of the study inclusion process.

Table 1. Detailed table of articles included (n=6).

Detailed information of included articles					
Authors/ Year/ Country	Study type and methodological quality (MMAT)	N° and characteristic of the population	Main exclusion criterion(a)	Follow-up	Intervention
Allan et al.[11]; 2019. Canada	Unblinded randomized clinical trial; 75%	35 women participated, aged over 35 years, and diagnosed with mild to moderate SUI	Pregnant women who gave birth or breastfed in the last 6 months; Women with abnormal pelvic examination, with pelvic organ prolapse greater than grade II	6-month follow-up, completed by 29 women	Monopolar radiofrequency; 220 pulses of 90 J/cm; Group 1: 1 session; Group 2: 2 sessions
Allan et al.[12]; 2020. Canada	Unblinded randomized clinical trial; 25%	35 women, over 35 years of age, and diagnosed with mild to moderate SUI participated in the study	Pregnant women who gave birth or breastfed in the last 6 months; Women with abnormal pelvic examination, with pelvic organ prolapse greater than II	12-month follow-up, completed by 25 women	Monopolar radiofrequency; 220 pulses of 90 J/cm; Group 1: 1 session; Group 2: 2 sessions
Lordelo et al.[13]; 2017. Brazil	Non-randomized clinical trial; 50%	10 women, between 43 and 66 years old, participated, with SUI being the main complaint reported	Women with organ prolapse; Chronic degenerative neurological diseases; Intrauterine copper device; Pacemaker; Pregnant women; Submitted to previous treatment for SUI	Follow-up, 1, 2, and 3 months after the end of the treatment	Non-ablative radiofrequency, Spectra G2 – Tonederm® in the external urethral meatus; 5 sessions, with weekly frequency; Temperatures ranging from 39–41°C
Caruth et al.[15]; 2018. USA.	Prospective clinical trial; 50%	30 women participated, between 40 and 60 who reported weakness in the PFM and vaginal flaccidity	Presence of implanted metallic or electronic device; Current or history of cancer; Pregnancy or lactation; Use of hormone replacement therapy	2-month follow-up after treatment	Bipolar device based on continuous or fractional radiofrequency; Cutting temperature of 43°C; 1 session;
Leibaschoff et al.[14]; 2016. Argentina.	Randomized, double-blind, controlled, and descriptive clinical trial; 25%	20 postmenopausal women with symptoms of SUI and vaginal laxity participated in the study	Women who had undergone surgery before; Recurrent presence of urinary tract infection; BMI >35	12 weeks of follow-up	Used ThermiVa ®; 3–5 min per area, with T=40–45°C; total ≥30 min; 1 treatment every 30 days for 3 months
Slongo et al.[16]; 2021. Brazil.	Randomized clinical trial; 100%	117 women, 45–65 years of age, complaining of SUI or MUI, with a predominance of stress, participated in the study	Stages III and IV pelvic organ prolapse; History of previous surgery for prolapse or UI; TMAP in the last 12 months; Hormone replacement in the last 6 months	3 months of intervention, no follow-up, only evaluation after one month of intervention	Microablative fractional radiofrequency was configured at 45 W and 4 MHz to power 64 microneedles, 0.2 mm in length, allocated in an area of 8 mm × 8 mm; RF applied monthly for three months; TMAP – 12 weekly sessions, lasting for 60 min

BMI: body mass index; UI: urinary incontinence; SUI: stress urinary incontinence; IUM: mixed urinary incontinence; MAP: pelvic floor muscles; RF: radiofrequency; TMAP: pelvic floor muscle training.

In contrast, another study[16] found that the 1-h Pad test had a mean significant decrease of 7.22 g after treatment in all groups (p<0.001) but no differences between them (p=0.987). However, the authors did not justify why the results were similar.

Secondary outcomes

Secondary outcomes were divided into urinary and non-urinary variables. Among the urinary variables, the urinary loss was recorded by the voiding diary[11,12], and the impact of UI on quality of life was evaluated using the following questionnaires: Incontinence Questionnaire-Urinary Incontinence Short Form (ICIQ-UI-SF)[14-16], Incontinence Impact Questionnaire Short Form (IIQ-7)[11,12,15], and Urogenital Distress Inventory (UDI-6)[11,12,14].

In their first study, Allan et al.[11] reported an 80% decrease in leakage episodes in both groups according to the 7-day voiding diary. A sustained improvement was observed for 6 months in relation to the subjective measures, based on the UDI-6, IIQ-7, and ICIQ-UI-SF questionnaires, compared to the initial assessment. In a second study[12], 64% of randomized subjects reported fewer episodes of leakage compared to that at the baseline. They also showed a decrease in UI symptoms and an improvement in quality of life through the UDI-6 and ICIQ-UI-SF questionnaires.

Regarding the use of questionnaires, Slong et al.[16] observed that there was a significant improvement between the groups in the evaluation of the ICIQ-UI-SF, highlighting a better result in the RF group with TMAP, which evolved from 13.6±3.8–8.2±5.2.

In Caruth's study[15], there was an improvement in the impact of UI (62.7%) and quality of life (64.6%) in the 2-month evaluation using the IIQ-7 and ICIQ-UI-SF. Leibaschoff et al.[14] observed a significant difference between the control (17.3±0.78) and active (11.4±0.66) groups in relation to the ICIQ-UI-SF. The UDI-6 also showed an improvement between groups (33.7±12.5 *versus* 16.2±6.0).

The so-called non-urinary outcomes considered the effects of RF in relation to changes related to the vaginal epithelium, vaginal dryness, and sexual function. However, not all studies evaluated these parameters, but those that covered these variables used the vaginal health index (VHI)[14,16] and ICIQ Vaginal Symptoms Questionnaire (ICIQ-VS)[15,16]. Some studies included the impact of pelvic organ prolapse and the impact of anorectal and urinary symptoms on patients' quality of life, using the Pelvic Floor Impact Questionnaire (PFIQ-7)[15].

To assess vaginal and sexual symptoms and quality of life, Leibaschoff et al.[14] showed differences between the control group and the active group. There was a significant improvement in the active treatment group, from 11.5±0.67–19.3±2.01, relative to VHI.

An author[15] observed an improvement of 50.6% for vaginal symptoms, 72.1% for sexual issues, and 61.2% for pelvic floor impact on reassessment after 2 months of intervention in the ICIQ-VS and PFIQ-7 assessments. Other authors[16] also found improvement in the ICIQ-VS after treatment in all groups, with the greatest improvement in the RF group (-9), followed by the RF+TMAP (-4.4) and TMAP groups (-3.4). The same authors also found improvement in vaginal moisture, fluid volume, vaginal pH, and elasticity only in the RF and RF+TMAP groups but with no difference between them. It was also observed that the epithelial integrity improved in all groups and vaginal dryness improved in the RF group. In terms of VHI, the RF+TMAP (+3.2) and RF (+2.9) groups were superior to the TMAP group (+0.5). Regarding flaccidity, there was no significant difference between the groups.

Adverse effects

No serious or unexpected adverse effects were observed[11-16]. Lordelo et al.[13] reported the presence of burning soon after the menstrual period in one patient; however, the physical examination did not show any change. Slongo et al.[16] described that a participant in the RF group had mild vaginal burns with spontaneous improvement and mild dyspareunia after three months.

DISCUSSION

UI is a public health problem, with varied treatments. Of these, surgical treatment is an option, which may be associated with complications and recurrences[17]. Other therapies can be recommended according to the nature and intensity of the UI, such as TMAP, which is a conservative treatment option[18]. The growing search for safe, non-invasive, and effective alternatives has become increasingly popular, emphasized review studies like this.

RF therapy has been widely used to treat dermatological and gynecological conditions. The microablative fractional-type RF, which is used to improve skin, vaginal, and vulvar mucosal trophism[19], results in promising responses in neocollagenesis and neoelastinogenesis[20,21], consequently leading to clinical improvement. Thus, it is observed that this therapy provides a less invasive treatment for women with SUI and MUI[11-16].

The importance of objective data evaluations is known, and thus, more accurate and coherent results can be obtained with the research. However, only four studies used the 1-h pad test to measure urine volume. Regarding secondary outcomes, two studies[13,16] evaluated PFM through digital palpation quantified by the modified Oxford scale. Another study[14] performed the

cytological analysis through a biopsy. Other studies[11,12,15] performed the evaluation using questionnaires.

Importantly, the main objective of this review was to evaluate the application of RF in relation to urine loss and not factors related to intimate esthetics and sexuality. All studies addressed patients diagnosed with SUI, and only one[17] included MUI in the sample.

Only two[12,16] studies showed a longer period of intervention. It is believed that a short period of intervention may not be able to assess the expected changes in neocollagenesis and neoelastinogenesis[20,21] and thus may not provide reliable results. Regarding sampling, most studies[11-15] recruited a small number of volunteers, and only one had more than 100 patients in the sample[16].

This review had some limitations. The studies that were included had low strength, lack of blinding, short post-intervention follow-up, and lack of clear description of randomization. Despite these limitations, it was observed that RF is an alternative therapy, proving to be effective and safe for the issues evaluated, which, according to the studies, is an alternative for the conservative management of patients with SUI.

CONCLUSIONS

The studies included in this review showed significant results of RF to resolve or minimize the complaints of women with SUI; however, according to the MMAT, the methodological quality of the studies was low. Therefore, more randomized, controlled, and blinded clinical trials are needed to provide safe therapy.

AUTHORS' CONTRIBUTIONS

MLAS: Investigation, Writing – review & editing. **MAPSS:** Investigation, Writing – review & editing. **FCR:** Formal Analysis, Writing – review & editing. **GPMG:** Supervision, Writing – review & editing. **LSE:** Supervision, Writing – review & editing. **MTABCM:** Supervision, Writing – review & editing.

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REVIEW ARTICLE
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Bacterial infections in COVID-19 patients: a review

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INTRODUCTION

The COVID-19 pandemic is the most severe transmissible event to affect the global population in more than a hundred years[1]. Along with the direct social and health-related consequences, the infection brought several indirect effects, that is, one of those is the increasing occurrence of bacterial resistance[2-4]. This effect was not only a direct consequence of the increase of antimicrobial consumption, mainly related to the real occurrence of bacterial infections, but also as a consequence of uncertainties due to the severity of COVID-19 infections and the difficulties in establishing a correct diagnosis of a concomitant or secondary bacterial infection. Additionally, adding up to these uncertainties and the increased prescription of antimicrobials, bacterial infections may worsen COVID-19 prognosis and viral infections are commonly perceived as risk factors for concomitant or subsequent bacterial infections.

There are two types of bacterial infections associated with COVID-19 or other viral infections[5]: (a) coinfections are the result of impaired immune systems, increased nasopharyngeal colonization, and damage of the respiratory tract mucosa, occurring at the same time or shortly after the appearance of COVID-19 or other viral symptoms, and (b) superinfections, which are usually healthcare-associated infections (HAIs), with clinical manifestation and diagnostic criteria resembling other HAIs and usually occurring in patients with severe COVID-19 submitted to invasive procedures during hospitalization.

Frequently, the clinical diagnosis of COVID-19-associated bacterial infections does not meet the criteria of coinfections or superinfections and is usually guided by clinical severity status or by previous experience, non-evidence-based. Such a situation leads to antibiotic misuse, ecological pressure, and previsible increase in bacterial resistance[6,7].

The objective of this study was to review the different aspects of the association of bacterial infections and COVID-19, namely, the impact of COVID-19 in antimicrobial use, incidence and etiology of bacterial infections associated with COVID-19, diagnostic strategies for bacterial infections in COVID-19, and antimicrobial stewardship strategies in COVID-19 patients.

COVID-19, VIRAL SEPSIS, AND ANTIMICROBIAL USE

COVID-19 is not only a respiratory infection but also a systemic infection[8]. After reaching the circulatory system, the severe acute respiratory syndrome *coronavirus 2 (*SARS-CoV-2) disseminates to several organs, using the angiotensin-converting enzyme-2 receptor (ACE-2) to enter the cell[9]. It infects not only the lungs but also several other cells such as enterocytes, renal cells, hepatic cells, and many others[10]. In severe cases, COVID-19 is associated with a cytokine storm, a hyperinflammatory syndrome that resembles bacterial sepsis, with multiorgan failure and an increase of inflammatory biomarkers[11,12]. This syndrome is related to viral subtypes, clinical predisposing factors, and host expression of variant immune proteins, such as toll-like receptors, human leukocyte antigen (HLA)[13], and ABO system[14].

The clinical manifestations of this syndrome are fever, dyspnea, hypotension, tachycardia, confusion, cough, oliguria, and other signs that are commonly seen in bacterial sepsis[8]. Under these circumstances, it is expected that the clinical differentiation between bacterial and viral sepsis is challenging.

The clinical use of antibiotics in COVID-19 is reportedly elevated[15], with an increase in the prescriptions of antibacterials and antifungals, potentially targeting secondary infections. Of note, the increased use of azithromycin as a potential

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antiviral drug has been reported, despite evidence against its use in these situations[16].

Langford et al.[15] performed a systematic review of antibiotic use in COVID-19 patients and found that 74.6% received an antibacterial agent. This proportion of patients receiving is substantially higher than in surgical or medical wards, whereas Charani et al. found the rates of 55 and 45%, respectively[17]. These rates vary according to the country, hospital, and characteristics of the patients. Antibiotic consumption is directly associated with the elevation of resistance rates[7].

There is a concern about the increase in antibiotic resistance as a consequence of the elevated rates of antibiotic prescriptions during the COVID-19 pandemic[18], as well as the reports of the occurrence of infections that may be associated with antibiotic use, such as *Clostridioides difficile*[19] or fungal infections[20,21].

INCIDENCE OF ASSOCIATED BACTERIAL INFECTIONS IN COVID-19

Contrasting with high antibiotic consumption, systematic studies show a low incidence of bacterial coinfections and superinfections in COVID-19 patients[22], with lower rates than the ones reported with influenza[23].

Two meta-analyses were performed on the incidence of bacterial infections in COVID-19 patients (Table 1). Langford et al.[24] analyzed 24 studies from 1,308 publications reviewed. The pooled bacterial infection incidence was 6.9% (95%CI 4.3–9.5), with a higher incidence in severely ill patients. They found 5.9% (95%CI 3.8–8.0) coinfected among all hospitalized patients and 8.1% in critically ill patients (95%CI 2.3–13.8). Coinfection was present in 3.5% (95%CI 0.4–6.7)[24] of COVID-19 patients at the time of initial clinical presentation, while superinfections were detected in 14.3% (95%CI 9.6–18.9)[24]. The latter were probably HAIs related to the use of antibiotics, invasive devices, and severity, and their incidence varied according to the characteristics of the hospital and patients[25]. Ventilator-associated pneumonia (VAP) was reported as the most frequent superinfection.

Table 1. Incidence of bacterial infections in COVID-19 patients.

Study	Infection	Incidence (%)	95%CI
Langford et al.[24]	Pooled rate	6.9	4.3–9.5
	Hospitalized patients	5.9	3.8–8.0
	ICU patients	8.1	2.3–13.8
	Coinfection	3.5	0.4–6.7
	Superinfection	14.3	9.6–18.9
Lansbury et al.[26]	Coinfection	7	3–12
	ICU patients	14	5–26
	Mixed hospital–ICU patients	4	1–9

CI: confidence interval; ICU: intensive care unit.

Lansbury et al.[26] analyzed 3,834 patients from 30 studies and considered only the laboratory-confirmed coinfections at the time of presentation. They found coinfection in 7% of patients (95%CI 3–12), and the subgroup analysis disclosed 14% (95%CI 5–26) in intensive care unit (ICU) and 4% (95%CI 1–9) in mixed hospital–ICU patients.

There is heterogeneity in the diagnostic criteria of the studies included in both meta-analyses. Nevertheless, both show low rates of coinfections and superinfections. The incidence of bacterial infections remains low even in studies of the autopsy findings including the most severe cases, where coinfections and superinfections could be expectedly higher. Clancy et al.[27] performed a systematic review including 621 patients from 75 studies focusing on histopathological criteria. Bacterial infections, including both coinfections or superinfections, were observed in 200 (32%) patients. The most common infection observed was pneumonia (95%), followed by abscesses or empyema (3.5%) and septic emboli (1.5%).

ETIOLOGY OF ASSOCIATED BACTERIAL INFECTIONS IN COVID-19

The etiology of coinfections and superinfections is also variable, depending on the clinical scenario. Studying coinfections, Lansbury et al.[26] showed that *Mycoplasma pneumoniae* was the most common agent and surprisingly *Streptococcus pneumoniae* was not identified in any patient, which probably indicates a selection or sample bias. Another bias in this study was the overexpression of *Pseudomonas aeruginosa* as a causative agent of community-acquired infections. This microorganism, frequently associated with healthcare-related infections, was more frequently isolated than the common causative agents of community-acquired infections, such as *S. pneumoniae* or *Haemophilus influenzae*. This finding probably indicates a selection bias, difficulties in discrimination between community- or healthcare-acquired infections, or selective use of diagnostic tools, such as bronchoscopy, in the more critically ill patients. This finding needs to be further clarified.

Singh et al.[28] used real-time polymerase chain reaction (PCR) in 50,419 individual samples for identifying the presence of SARS-CoV-2 and other bacterial and viral respiratory pathogens, as an effort to evaluate coinfections in COVID-19 patients. From 4,259 SARS-CoV-2-positive patients, bacterial

agents were detected in 33%, with *S. pneumoniae* (8.66%), *H. influenzae* (9.27%), and *Staphylococcus aureus* (13.17%) being the most frequently identified.

Sharov et al.[29] studied bacterial coinfections during the initial epidemic. They analyzed 3,382 samples and similarly identified *S. pneumoniae*, *S. aureus*, and *H. influenzae* as the most common agents associated with bacterial pneumonia.

No studies have evaluated the agents associated with HAIs, especially VAP, in COVID-19 patients; it should, however, be noted that the etiology of such superinfections is highly dependent on local epidemiology. Nevertheless, the increased incidence of *Acinetobacter baumannii* and the fungal infections has been reported[27], which has not been detected in all locations and may reflect local characteristics.

As for the occurrence of resistant bacteria, they were reportedly low in coinfections, which is at least partly explained by the absence of previous antibiotic exposure or hospital contact in many COVID-19 patients. This lack of risk exposure may lead to a lower risk of nasopharyngeal colonization by drug-resistant bacteria such as *P. aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA). Finally, the current data do not support the empirical use of broad-spectrum agents in the treatment of coinfections, particularly in patients without risk factors for antibiotic resistance.

DIAGNOSIS OF BACTERIAL INFECTIONS IN COVID-19

While the diagnosis of VAP or HAI may follow previously established criteria, the diagnosis of coinfections is particularly difficult, once severe COVID-19 may present as sepsis, resembling many aspects of a bacterial etiology. There is no unique clinical sign or laboratory test sufficiently specific to discriminate between viral and bacterial etiology in COVID-19 patients with sepsis.

Since severe COVID-19 may be considered a viral sepsis, many clinical features such as high fever and signs of organ failure may be present[8,30-32], making differentiation between single-agent infection and coinfection a difficult task. New-onset and high fever may be an indication of the development of a coinfection[33] but may also reflect the worsening of clinical status and cytokine storm. An important aspect is that cough in COVID-19 cases is more frequently nonproductive, and bacterial coinfection may commonly present with productive cough and purulent sputum.

Radiologic features are not either completely specific. Both bacterial and viral pneumonia may generate consolidative foci. COVID-19 consolidations in images usually develop in late disease phases and are characterized by multiple peripheral consolidations, while bacterial pneumonia tends to be single and accompanied by air bronchograms[34].

Laboratory tests and biomarkers have been proposed as an important laboratory aid to clinical diagnosis. Neutrophilia, lymphopenia, increased neutrophil-to-lymphocyte rate, thrombocytopenia, elevated transaminases, and lactic dehydrogenase may be useful for the establishment of prognosis but do not discriminate between bacterial and viral infections[35-37].

C-reactive protein (CRP) and procalcitonin have been proposed as the indicators of a bacterial coinfection, but both CRP and procalcitonin do not reach an acceptable specificity to be useful confirmatory tests of bacterial infections[38]. In fact, both biomarkers may be elevated during cytokine storm[37,39-41], blurring their positive predictive values. Dolci et al.[42] studied the value of biomarkers in 83 COVID-19 patients and 33 of those with bacterial secondary infections. Procalcitonin and CRP had a low accuracy (area under receiver-operating characteristic curve [AUC]: 0.757 and 0.874, respectively) and also a weak positive predictive value (0.650 and 0.654, respectively). These findings led to the deduction that the use of both biomarkers without more discriminant, associated clinical data may lead to the unnecessary prescription of antibiotics. However, they are specific enough to help rule out bacterial infections, in conjunction with other clinical and radiological findings[43].

ANTIMICROBIAL STEWARDSHIP STRATEGIES IN COVID-19

Improving antimicrobial prescriptions in a COVID-19 scenario may focus not only on reducing the consumption of broad-spectrum antibiotics or the duration of antibiotic therapy but also on the development of more specific diagnostic criteria for bacterial infections, which may reflect in the general amount of antibacterial prescriptions[44].

A combination of clinical and subsidiary data may be more useful for the diagnosis of bacterial infection than using a single biomarker alone. Some studies[45] suggested that a clinical score alone or combination of data including biomarkers may reduce antimicrobial consumption. This combination may consider the worsening of the clinical status; appearance of a new, high-degree fever and purulent sputum; image with central consolidations or air bronchogram; worsening of lymphopenia; and increased biomarkers such as CRP, procalcitonin, or interleukin-6[33]. Peters et al.[45] proposed a clinical pathway, which may be useful as a guide for specialists and nonspecialists to drive into a better antibiotic prescription (Figure 1). A combination of clinical history and physical examination and procalcitonin may probably be more accurate than a single marker for indicating antibiotic therapy, and clinical reasoning is an important tool in antimicrobial stewardship.

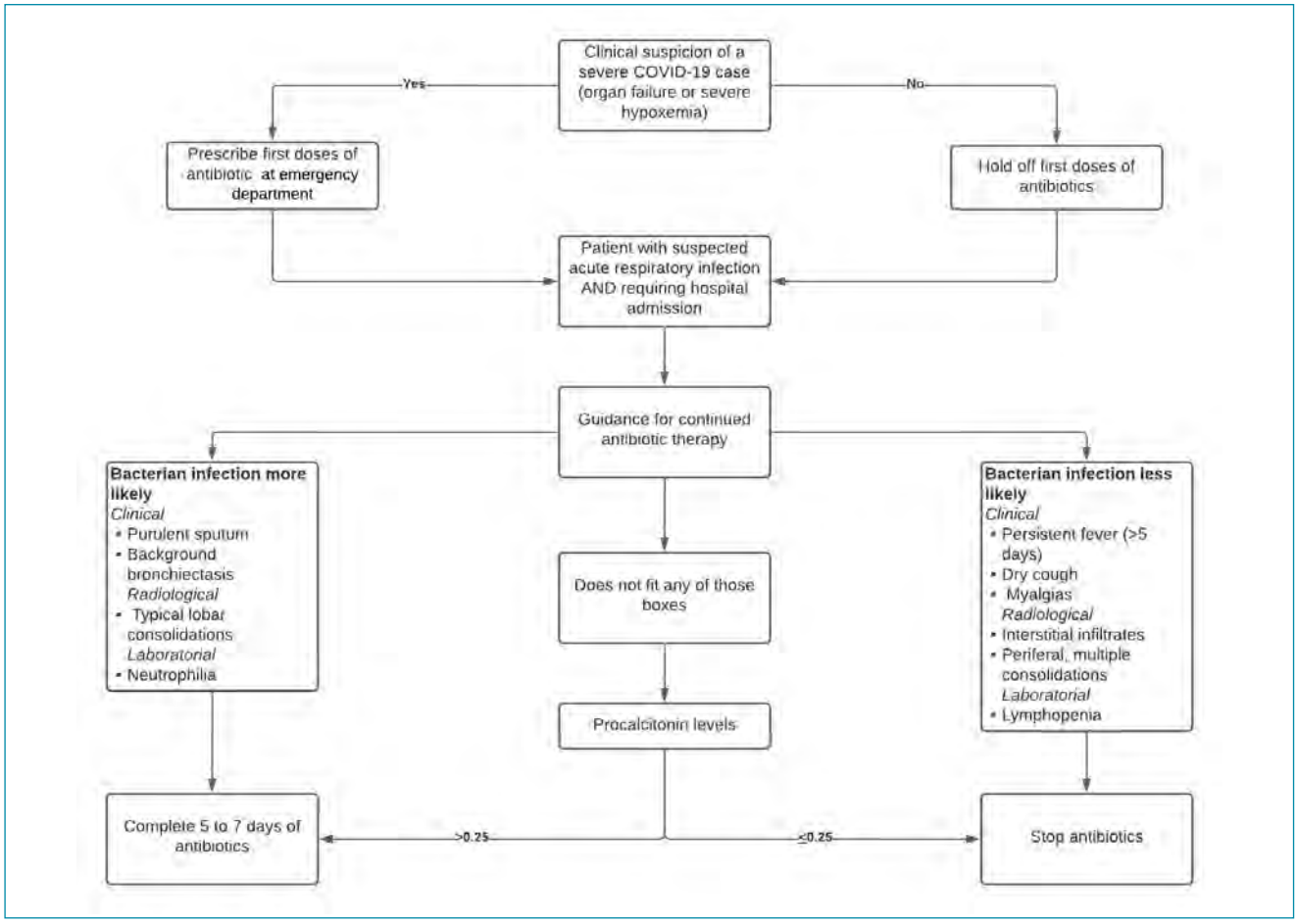

Figure 1. Clinical pathway to guide antibiotic therapy in COVID-19 patients. Adapted from Peters et al.[45].

CONCLUSIONS

Our review reinforces that bacterial coinfections are uncommon in COVID-19 settings, and there is not a single clinical finding, radiological, or laboratory biomarker that is sufficiently specific to guide diagnosis. A combination of signs and tests may be more discriminant than a single marker alone. These efforts could lead to a decrease in antibiotic prescription and consumption with potential improvement in resistance emergence and clinical outcomes. The development of more specific clinical criteria or score is a priority and may help improve clinical practice for COVID-19 or any other viral respiratory infection guidelines.

AUTHORS' CONTRIBUTION

RSG: Writing – original draft, Writing – review & editing.
CRVK: Writing – original draft, Writing – review & editing.

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COMMENTARY
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Comment on "Hematological detraining-related changes among elderly individuals with high blood pressure"

Ximei Qi[1], Qiang Huang[1], Xinyan Qi[1], Zhonglan Li[1], Fan Wang[1]*

Dear Editor,

We read the recent article titled "Hematological detraining-related changes among elderly individuals with high blood pressure" published by Cancela et al.[1] with great interest. The study found that the number of minutes/week of aerobic and resistance exercise training over 18 non-consecutive months was not a significant determinant factor in the development of hypertension during three months of detraining. However, some issues should be raised from our point of view.

First, it is not a reasonable grouping for weekly exercise time. In the study, subjects were divided into two groups, depending on whether the amount of workout in minutes/week was higher (G>150) or lower (G<150) than 150 min/week. Significant differences were noted in weight and body mass index (BMI). In sum, baseline characteristics were not comparable between the two groups.

Second, the comparison of the total cholesterol, glucose, insulin, and weight before and after aerobic and resistance exercise is more reasonable in our opinion. To evaluate the effect of supervised progressive resistance exercise training on total cholesterol, glucose, insulin, weight, and other parameters, all available subjects should be included in the analysis.

AUTHORS' CONTRIBUTIONS

XQ: Data curation, Formal Analysis, Writing – original draft. **QH:** Data curation, Formal Analysis, Writing – original draft. **XQ:** Data curation, Formal Analysis, Writing – original draft. **ZL:** Data curation, Formal Analysis, Writing – original draft. **FW:** Conceptualization, Writing – review & editing.

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ERRATUM

https://doi.org/10.1590/1806-9282.67.Suppl1.20200721ERRATUM

In the manuscript "COVID-19 pandemic information on Brazilian websites: credibility, coverage, and agreement with World Health Organization. Quality of COVID-19 online information in Brazil " , DOI: 10.1590/1806-9282.67.Suppl1.20200721, published in the Rev Assoc Med Bras. 2021;67(Suppl 1):57-62:

Page 57, title:

Where it reads:

COVID-19 pandemic information on Brazilian websites: credibility, coverage, and agreement with World Health Organization. Quality of COVID-19 online information in Brazil

It should read:

COVID-19 pandemic information on Brazilian websites: credibility, coverage, and agreement with World Health Organization

Page 61, Table 3:

Where it reads:

Table 3. Quality content analysis of the individual websites.

Content analysis, n (%)	*Websites*									
	bbc.com/		coronavirus.pr.gov.br/	coronavirus.saude.gov.br/	dasa.com.br/coronavirus	especiais.gazetadopovo.com.br/coronavirus/	especiais.g1.globo.com/	estadao.com.br	folha.uol.com.br/	goiania.go.gov.br/
Overall										
Total	14 (46.7)	18 (72.0)	5 (55.6)	17 (54.9)	18 (60.0)	19 (63.4)	31 (77.5)	18 (56.3)	18 (51.5)	8 (44.5)
Partial	14 (46.7)	7 (28.0)	1 (11.1)	11 (35.5)	10 (33.3)	10 (33.3)	9 (22.5)	12 (37.5)	14 (40.0)	8 (44.4)
Disagreement	2 (6.6)	0 (0.0)	3 (33.3)	3 (9.6)	2 (6.7)	1 (3.3)	0 (0.0)	2 (6.2)	3 (8.5)	2 (11.1)
Definition (0–2)										
Total	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)
Partial	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Symptoms (0–4)										
Total	1 (25.0)	3 (75.0)	0 (0.0)	2 (50.0)	1 (25.0)	2 (50.0)	2 (66.7)	0 (0.0)	2 (50.0)	1 (25.0)
Partial	3 (75.0)	1 (25.0)	0 (0.0)	1 (25.0)	3 (75.0)	2 (50.0)	1 (33.3)	3 (75.0)	2 (50.0)	1 (25.0)
Disagreement	0 (0.0)	0 (0.0)	2 (100.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	2 (50.0)
Spreading (0–9)										
Total	1 (20.0)	3 (100.0)	1 (100.0)	2 (66.7)	3 (75.0)	2 (66.7)	5 (71.5)	5 (83.4)	1 (16.6)	1 (50.0)
Partial	3 (60.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (25.0)	1 (33.3)	2 (28.5)	0 (0.0)	5 (83.4)	1 (50.0)
Disagreement	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.6)	0 (0.0)	0 (0.0)
Prevention (0–17)										
Total	6 (60.0)	7 (70.0)	4 (80.0)	8 (66.6)	7 (63.7)	6 (60.0)	10 (90.9)	7 (63.7)	4 (40.0)	6 (66.7)
Partial	4 (40.0)	3 (30.0)	1 (20.0)	2 (16.7)	3 (27.3)	4 (40.0)	1 (9.1)	4 (36.3)	4 (40.0)	3 (33.3)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)	1 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)
Treatment (0–7)										
Total	2 (100.0)	1 (50.0)	0 (0.0)	2 (100.0)	2 (100.0)	2 (100.0)	3 (75.0)	2 (100.0)	2 (66.7)	0 (0.0)
Partial	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (33.3)	2 (100.0)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others (0–18)										
Total	4 (44.4)	2 (50.0)	0 (0.0)	3 (37.5)	3 (42.9)	5 (55.6)	9 (69.3)	2 (28.5)	7 (70.0)	0 (0.0)
Partial	4 (44.4)	2 (50.0)	0 (0.0)	5 (62.5)	3 (42.9)	3 (33.3)	4 (30.7)	5 (71.5)	2 (20.0)	1 (100.0)
Disagreement	1 (11.2)	0 (0.0)	1 (100.0)	0 (0.0)	1 (14.2)	1 (11.1)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)

It should read:

Table 3. Individual website quality content analysis.

Content analysis, n (%)	Websites									
	bbc.com/	brasilescola.uol.com.br/	coronavirus.pr.gov.br/	coronavirus.saude.gov.br/	dasa.com.br/coronavirus	especiais.gazetadopovo.com.br/coronavirus/	especiais.g1.globo.com/	estadao.com.br	folha.uol.com.br/	goiania.go.gov.br/
Overall										
Total	14 (46.7)	18 (72.0)	5 (55.6)	17 (54.9)	18 (60.0)	19 (63.4)	31 (77.5)	18 (56.3)	18 (51.5)	8 (44.5)
Partial	14 (46.7)	7 (28.0)	1 (11.1)	11 (35.5)	10 (33.3)	10 (33.3)	9 (22.5)	12 (37.5)	14 (40.0)	8 (44.4)
Disagreement	2 (6.6)	0 (0.0)	3 (33.3)	3 (9.6)	2 (6.7)	1 (3.3)	0 (0.0)	2 (6.2)	3 (8.5)	2 (11.1)
Definition (0–2)										
Total	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)
Partial	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Symptoms (0–4)										
Total	1 (25.0)	3 (75.0)	0 (0.0)	2 (50.0)	1 (25.0)	2 (50.0)	2 (66.7)	0 (0.0)	2 (50.0)	1 (25.0)
Partial	3 (75.0)	1 (25.0)	0 (0.0)	1 (25.0)	3 (75.0)	2 (50.0)	1 (33.3)	3 (75.0)	2 (50.0)	1 (25.0)
Disagreement	0 (0.0)	0 (0.0)	2 (100.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	2 (50.0)
Spreading (0–9)										
Total	1 (20.0)	3 (100.0)	1 (100.0)	2 (66.7)	3 (75.0)	2 (66.7)	5 (71.5)	5 (83.4)	1 (16.6)	1 (50.0)
Partial	3 (60.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (25.0)	1 (33.3)	2 (28.5)	0 (0.0)	5 (83.4)	1 (50.0)
Disagreement	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.6)	0 (0.0)	0 (0.0)
Prevention (0–17)										
Total	6 (60.0)	7 (70.0)	4 (80.0)	8 (66.6)	7 (63.7)	6 (60.0)	10 (90.9)	7 (63.7)	4 (40.0)	6 (66.7)
Partial	4 (40.0)	3 (30.0)	1 (20.0)	2 (16.7)	3 (27.3)	4 (40.0)	1 (9.1)	4 (36.3)	4 (40.0)	3 (33.3)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)	1 (9.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)
Treatment (0–7)										
Total	2 (100.0)	1 (50.0)	0 (0.0)	2 (100.0)	2 (100.0)	2 (100.0)	3 (75.0)	2 (100.0)	2 (66.7)	0 (0.0)
Partial	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	1 (33.3)	2 (100.0)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others (0–18)										
Total	4 (44.4)	2 (50.0)	0 (0.0)	3 (37.5)	3 (42.9)	5 (55.6)	9 (69.3)	2 (28.5)	7 (70.0)	0 (0.0)
Partial	4 (44.4)	2 (50.0)	0 (0.0)	5 (62.5)	3 (42.9)	3 (33.3)	4 (30.7)	5 (71.5)	2 (20.0)	1 (100.0)
Disagreement	1 (11.2)	0 (0.0)	1 (100.0)	0 (0.0)	1 (14.2)	1 (11.1)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)

Content analysis, n (%)	Websites									
	hospitalsiriolibanes.org.br/	istoe.com.br/	metropoles.com/	portal.anvisa.gov.br/	prefeitura.pbh.gov.br/	rededorsaoluiz.com.br/	saopaulo.sp.gov.br/	sergiofranco.com.br/	unimedpoa.com.br/	uol.com.br/
Overall										
Total	23 (79.4)	14 (66.6)	5 (41.6)	10 (50.0)	5 (21.8)	11 (50.1)	6 (28.6)	5 (26.4)	23 (69.7)	21 (63.7)
Partial	4 (13.8)	7 (33.3)	6 (50.1)	8 (40.0)	16 (69.6)	10 (45.4)	11 (52.4)	11 (57.9)	10 (30.3)	10 (30.3)
Disagreement	2 (6.8)	0 (0.0)	1 (8.3)	2 (10.0)	2 (8.6)	1 (4.5)	4 (19.0)	3 (15.7)	0 (0.0)	2 (6.0)
Definition (0–2)										
Total	2 (100.0)	1 (100.0)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)	2 (100.0)
Partial	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (100.0)	1 (50.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Disagreement	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Symptoms (0–4)										
Total	2 (50.0)	1 (50.0)	0 (0.0)	1 (25.0)	0 (0.0)	2 (50.0)	0 (0.0)	0 (0.0)	3 (75.0)	0 (0.0)
Partial	2 (50.0)	1 (50.0)	1 (100.0)	2 (50.0)	3 (100.0)	2 (50.0)	2 (66.7)	1 (50.0)	1 (25.0)	4 (100.0)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (50.0)	0 (0.0)	0 (0.0)
Spreading (0–9)										
Total	5 (83.4)	2 (66.7)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (50.0)	2 (40.0)
Partial	1 (16.6)	1 (33.3)	0 (0.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	2 (66.7)	3 (50.0)	3 (60.0)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
Prevention (0–17)										
Total	8 (72.8)	5 (71.5)	2 (40.0)	5 (55.6)	4 (40.0)	6 (66.7)	2 (28.5)	1 (25.0)	7 (77.8)	7 (70.0)
Partial	1 (9.0)	2 (28.5)	3 (60.0)	3 (33.3)	5 (50.0)	3 (33.3)	3 (42.8)	3 (75.0)	2 (22.2)	2 (20.0)
Disagreement	2 (18.2)	0 (0.0)	0 (0.0)	1 (11.1)	1 (10.0)	0 (0.0)	2 (28.5)	0 (0.0)	0 (0.0)	1 (10.0)
Treatment (0–7)										
Total	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	2 (66.7)	2 (66.7)	3 (100.0)
Partial	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (50.0)	1 (33.3)	1 (33.3)	0 (0.0)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others (0–18)										
Total	4 (100.0)	3 (50.0)	0 (0.0)	2 (100.0)	1 (20.0)	1 (50.0)	1 (25.0)	2 (33.4)	6 (66.7)	7 (77.8)
Partial	0 (0.0)	3 (50.0)	1 (100.0)	0 (0.0)	3 (60.0)	1 (50.0)	2 (50.0)	3 (50.0)	3 (33.3)	1 (11.1)
Disagreement	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	1 (25.0)	1 (16.6)	0 (0.0)	1 (11.1)

Note: The level of websites in agreement with the WHO items is expressed overall and per categories.