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Relevant aspects of acute appendicitis

Andy Petroianu[1]*

INTRODUCTION

Appendicitis is the most frequent abdominal surgical emergency. The risk of developing an appendiceal disorder during life is 7%, which represents 11 cases per 10,000 inhabitants per year. It may occur at any age, but it is generally found in patients aged from 15 to 30 years (23 cases/10,000 inhabitants/year)[1-4]. The diversity of differential diagnoses makes the right-side acute abdominal pain more difficult to be elucidated, especially in women. Even when all symptoms and exams indicate appendicitis, the surgical procedure and the histopathological exam of the appendix may show a normal appendix, another diagnosis, or no abdominal disorder. Despite many thousands of published studies about appendicitis, the appendix is still a mysterious organ and its disorders are still not well known

RELEVANT ASPECTS

This critical literature review of acute appendicopathies, including appendicitis, emphasizes 10 pivotal topics, which are relevant to the diagnosis and treatment of appendiceal disorders. This systematic search of the literature was performed on PubMed and Medline according to the PRISMA.

ETIOPATHOGENESIS OF THE ACUTE APPENDICOPATHIES

The appendix is a misunderstood organ, considering the unidentified role it plays in the body. Despite the paracecal position, the appendiceal characteristics are different from all other digestive organs and do not seem to be linked to digestion[1,2]. Its rich neuroendocrine and immune cell structure, as well as its disorders, have a closer relationship with neuroendocrine and immune systems. To reinforce this concept, it is important to highlight that the most frequent cancer of the digestive system is adenocarcinoma, but the most common appendiceal malignancy is the carcinoid tumor, which belongs to the neuroendocrine system[2].

There are many theories about the pathogenesis of appendicitis, most of which are associated with obstructive factors and intraluminal hypertension, but none of them has been proven to be true in experimental and clinical studies[1-4]. Another theory describes the neuroimmunoendocrine appendicopathy, whose clinical picture is similar to that of acute appendicitis, but without inflammation[4-9]. This disease, known since the beginning of the past century, may clarify the morphologically normal appendices found in patients with clinical and radiological manifestations of acute appendicitis.

DIAGNOSIS OF ACUTE APPENDICITIS

The diagnosis of appendicitis is a challenge even for surgeons with great clinical experience. Migrating pain to the right flank associated with hyporexia, fever, and a painful mass in the right flank is the classic clinical manifestation of acute appendicitis. However, this medical condition, accompanied by leukocytosis (>10,000/mm^3), neutrophilia (70–95%), and toxic granulations in the leukocytes, is present in less than 60% of patients with acute appendicitis[2,10].

Abdominal radiography in the anteroposterior view presents disorders in up to 95% of the cases and discloses distension of the cecum and ascending colon with fecal accumulation in the cecum, due to an adynamic ileum, as a cecal response to the appendiceal inflammation[11-13]. Other radiographic findings are appendiceal fecaliths, air within the appendix, loss of the cecal wall and psoas muscle boundaries, and a nonspecific mass in the right flank[2,14,15]. The ultrasound exam shows a non-mobilized and enlarged (>6 mm) inflamed appendix[2,15,16]. Computed tomography presents findings similar to those found in radiography and ultrasound[2,15,16].

WHEN TO OPERATE AN ACUTE APPENDICITIS

Acute appendicitis diagnosed in a critically ill patient with clinical instability must be immediately operated due to the risk of

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serious complications that occur when appendicitis progresses to perforation, peritonitis, and sepsis[2,4]. In contrast, if the suspicion of acute appendicitis occurs in a young patient, who is previously healthy and with uncharacteristic symptoms, the patient may be hospitalized for more exams and clinical observation. In these cases, if the option is for surgical treatment, it can be performed during the day under favorable conditions for the patient and the surgical team[2,4].

SURGICAL ACCESS TO APPENDECTOMY

The current preference is for laparoscopy through three ports (umbilical and suprapubic of 10 mm and another in the right flank of 5 mm). This procedure initially allows for diagnostic confirmation of appendiceal inflammation. Even if the appendix looks normal, most surgeons opt for appendectomy. However, according to the surgeon's decision, laparoscopy may only be propaedeutic, without removing a morphologically normal appendix. In this case, the patient must be controlled and informed that he/she still has an appendix and should be kept in the hospital until the clinical manifestations disappear. If there is a worsening of the clinical manifestations without evidence of another disorder, the removal of the appendix is indicated through a second laparoscopy during the same hospitalization[1,4,17,18]. The main advantages of laparoscopy are as follows:

- more accurate diagnosis of the appendicopathy;
- access to the entire abdomen for diagnosis and treatment of another disorder, if the appendiceal inflammation is not confirmed;
- removal of the appendix inside the trocar, without contamination of the abdominal wall and, consequently, less risk of surgical infection;
- the possibility of other procedures in the same surgical act, such as aspiration of liquids, including pus, in generalized peritonitis, and drainage of abscesses;
- lower incidence of incisional hernias;
- less postoperative pain;
- faster return to normal activities.

Single-port appendectomy is performed by surgeons who have experience in laparoscopy and have adequate devices. Appendectomy using robotic arms associated with single port access will gain space in surgical procedures in the near future[19-21].

Laparoscopy may be contraindicated or converted to laparotomy in the presence of the following:

- cardiovascular collapse and pulmonary failure exacerbated by pneumoperitoneum;
- multiple abdominal adhesions resulting from previous surgical procedures or other abdominal disorders that prevent safe access to the appendix;
- a woman in the last trimester of pregnancy, due to difficulty in accessing the appendix, mainly by surgeons with less practice;
- difficulty to control a surgical complication.

Specific transverse incision over the McBurney's point is indicated if there is no possibility of laparoscopy or if the surgeon has no experience with this method. When performing a laparotomy, even with a specific incision, the appendix must always be removed, regardless of whether it is inflamed or not[1,4,18].

MANAGEMENT OF THE APPENDICEAL STUMP AND DRAINAGE

Before the laparoscopic appendectomy, the management of the appendiceal stump after the removal of the appendix was a controversial issue. Stump ligature with or without its invagination, and even leaving the stump without ligature but only with local drainage have been published[1,4,18]. However, experience has shown that there is no difference between the results of the different managements. In laparoscopy, the stump is only clipped or ligated without invagination or coverage and no relevant complication has been registered when the surgical procedure is adequate[1,4,18-21]. Therefore, the management of the appendiceal stump is up to the surgeons to use their own best judgment.

Another controversial issue is regarding the drainage of the operated region. Some surgeons routinely leave local drains; however, most of them prefer to drain when there is no conviction in the perfect closure of the appendiceal stump or cecum. The lack of consensus also refers to the type of drain to be used. Some opt for the rubber Penrose drain, others for a silicone tubular, multitubular, covered tubular, or even the combination of several drains. This difference in options is due to uncertain and unpredictable drainage efficacy. Even in the presence of multiple local drains, a cecal fistula may be externalized far from its origin without any drain path. In fact, fistulas remain a difficult challenge to be understood and prevented. The drain should be maintained only if the drainage volume is above 30 ml/day. The patient in good health can be discharged from the hospital and change the drain dressings at home or at a medical service. After the drain is removed, a new abdominal imaging study must be performed to confirm that there is no longer any collection to be drained.

CARE WITH THE SURGICAL ACCESS

Skin antisepsis before all surgical procedures, including appendectomies, should use soapy antiseptics and alcoholic solutions, preferably iodinated, for a sufficient time to achieve an adequate and effective cleaning. All studies show that care and well-done skin antisepsis is the most effective method of reducing surgical infection. Antibiotic prophylaxis may be recommended, although its results are not certain, and there is no uniformity regarding the drug and its period of use[1,4,18,19,22]. Protectors of the abdominal wall in appendectomies did not prove their efficacy.

At the end of laparoscopy operations, it is useless to clean the port, as the trocars protect it. In contrast, after appendectomy through laparotomy, the abdominal wall must be cleaned after closing the peritoneum. There is no consensus on the solution to be used, and comparative studies have shown no difference among them. Most surgeons use 0.9% saline solution at room temperature with good results; however, other solutions may be suitable as long as they do not harm the tissues[2,4].

POSTOPERATIVE ABDOMINAL COMPLICATIONS

Most of the appendectomy postoperative follow-ups are uneventful with progressive improvement of the patients, who may be discharged from the hospital on the first postoperative day. However, in the presence of fever, sickness, and abdominal pain, the patient must not be discharged from the hospital, and laboratory and imaging exams are mandatory. The most frequent complications include infection of the abdominal wall, pericecal abscess, and right subphrenic abscess. Appendiceal stump or cecal fistula may also occur, and the diagnosis can be confirmed by ultrasound or computed tomography. Immediate adequate drainage is required either for an intra-abdominal abscess or fistula, and the drain may be introduced using an imaging method. If the complication cannot be cured by conservative drainage, a new surgical treatment may be required. Using laparoscopy, most complications, including general peritonitis, are adequately treated. Surgical difficulties and complications indicate conversion to laparotomy[1,4,18-22].

NONOPERATIVE TREATMENT

Antibiotics and clinical control are the most widely recommended treatments in the presence of uncomplicated abdominal inflammatory diseases, such as diverticulitis, salpingitis, enterocolitis, and cholecystitis. Acute appendicitis has also been conservatively treated on special occasions over the past 70 years, with good immediate results in 60% of the cases[23-25]. The other 40% of the patients worsen their health condition or experience recurrence of symptoms during the early post-treatment period. All of these cases must be immediately submitted to appendectomy[23-25].

The conservative treatment started in the early years of the twentieth century for patients in adverse conditions, such as the absence of surgeons or an adequate surgical environment. The clinical results were considerably improved after the introduction of antibiotics. The conservative treatment starts with parenteral large-spectrum antibiotics against gram-negative bacteria. If the patients are in a good healthy condition, they may be discharged from the hospital and are advised to take the prescribed oral antibiotics for 2 weeks[23-25].

FOOD INTAKE

Until half a century ago, the food intake was progressively released from restricted liquid only after patients had efficacious peristalsis for fear of colonic stasis and consequent cecal fistula. However, all studies have shown that regular food intake can be released since the first postoperative day. Fistulas and abscesses are not related to food intake[17,22]. Major complications, such as peritonitis, or postoperative nausea and vomiting, usually due to the side effects of anesthetics, hinder the onset of food intake until the reduction of the patient's symptoms. During this short time, parenteral hydration is recommended[17,22].

RETURN TO USUAL ACTIVITIES AFTER TREATMENT

The patient should be discharged from the hospital as soon as he/she is able to walk and eat, and has a normal autonomy, even on the same day of the surgical procedure[2,14]. To avoid the risk of incisional hernia, regardless of the size of the operation and surgical access, abdominal efforts should be avoided for at least 60 days[17,22].

FINAL CONSIDERATIONS

Despite being studied in many thousands of published works, the appendix is still an unknown organ in terms of its characteristics and functions. The pathophysiology of appendicopathies, including acute appendicitis, has not yet been established. Surgical progress has made the treatment safer, with better results and a faster recovery of patients to their normal life.

AUTHORS' CONTRIBUTIONS

AP: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, original draft writing, review and editing.

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Endoscopic treatment of gastric antral vascular ectasia

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field, to standardize how to conduct, and to assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted depending on the conditions and the clinical condition of each patient.
Guideline conclusion: April 2021.
Societies: Sociedade Brasileira de Endoscopia Digestiva.

INTRODUCTION

Gastric antral vascular ectasia (GAVE) is usually a condition characterized by vascular lesions located in the antrum, typically leading to occult or overt bleeding. It accounts for up to 4% of all nonvariceal upper gastrointestinal bleeding and may be present in 6–14% of patients with cirrhosis[1,2]. Patients commonly present with chronic iron deficiency caused by anemia, and up to 62% of patients may become transfusion-dependent[3].

Although the exact pathogenesis is still unclear, it has been proposed that abnormal gastric contraction waves induce prolapse of the antral mucosa with intermittent obstruction of blood vessels, resulting in fibromuscular hyperplasia. The imbalance of vasoactive and angiogenic mediators, such as prostaglandin E2, vasoactive intestinal peptide, 5-hydroxytryptamine, and gastrin, may also be involved in the pathogenesis[4,5]. Cirrhosis, connective tissue disorders, end-stage kidney disease, and bone marrow transplantation are disorders associated with GAVE development. Diagnosis is usually established by esophagogastroduodenoscopy; however, uncertain cases require histological assessment[6,7]. Endoscopically, it may present with three different patterns: stripes radiating to the pylorus (classically called "watermelon stomach," more common in noncirrhotic patients), diffuse punctate lesions (more common in cirrhotic patients), and a nodular type[8,9]. Histological features of GAVE include tortuous and dilated mucosal capillaries, often occluded by thrombi, and dilated submucosal veins surrounded by fibrohyalinosis and fibromuscular hyperplasia. The main differential diagnosis is portal hypertensive gastropathy (PHG).

Regarding treatment options, pharmacological therapies, such as beta-blockers, octreotide, thalidomide, or tranexamic acid, provide less benefits[4,10-12]. Antrectomy has higher morbidity and mortality. Transjugular intrahepatic portosystemic shunt is also not an effective therapy[13]. Therefore, the mainstay of treatment for GAVE is endoscopic therapy.

Argon plasma coagulation (APC) is a noncontact technique that delivers high-frequency monopolar current through ionized argon gas, resulting in tissue coagulation with limited depth of injury and lower risk of complications[14,15]. Endoscopic band ligation (EBL), first described as a treatment for esophageal varices, was further introduced as a treatment of GAVE. It consists of mechanical strangulation of the lesions by multiple elastic bands placement, resulting in thrombosis, necrosis, and subsequent fibrosis of the mucosa and submucosa[16,17]. Heater probe, Nd-YAG laser, sclerotherapy, and cryotherapy have been largely replaced due to complications of lower success rates and/or availability issues[4]. More recently, radiofrequency ablation (RFA)[18,19] and hybrid-APC[20] have emerged as alternative therapies.

Despite considerable recurrence rates, APC remains the most widely used endoscopic treatment for GAVE, although EBL shows promising results. Our aim was to perform a systematic review and meta-analysis in order to evaluate the best treatment option for this condition.

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METHODS

We performed a comprehensive search in electronic databases (i.e., MEDLINE, Embase, Cochrane, and LILACS) and grey literature. Only randomized controlled trials (RCTs) comparing APC and EBL for the treatment of GAVE were included.

A systematic review and meta-analysis were performed according to PRISMA guidelines using the PICO system. The risk of bias was assessed by the Cochrane risk-of-bias tool for randomized trials (RoB2). We analyzed the risk of bias for each outcome of every included study. The quality of evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria. The statistical analyses were conducted using the RevMan software, version 5.4, exposing the results as forest plots.

RESULTS

The search strategy identified 5,587 articles. After the removal of duplicates, 1,478 articles were screened. Among them, 11 were eligible for full-text review. Five observational studies, one review, and one study with duplicate data were excluded. Four[21-24] randomized clinical trials met the inclusion criteria, totaling 204 patients and comparing EBL with APC (102 patients in each group) (Annex Figure 1). The baseline characteristics of the population and the results of individual studies are reported in Table 1. All included studies had a follow-up period of 6 months.

Risk of bias and quality of studies

The overall risk of bias is reported in Annex Table 1. We considered that the overall risk of bias was not serious. The quality of the included studies was considered adequate (Annex Table 2).

Endoscopic eradication

A total of 116 patients were analyzed from three studies[21,23,24]. EBL presented a higher endoscopic eradication rate compared with APC (risk difference [RD]: 0.29, 95% confidence interval (CI): [0.14, 0.44], p=0.0001, I^2=0%) (Annex Figure 2). The GRADE analysis revealed a high certainty of evidence.

Recurrence of bleeding

A total of 116 patients were analyzed from three studies[21,23,24]. EBL presented a less recurrence of bleeding compared with the APC group (RD: 0.29, 95%CI: [0.15, 0.44], p<0.0001, I^2=0%) (Annex Figure 3). The GRADE analysis revealed a high certainty of evidence.

Table 1. Baseline characteristics of the population and the results of individual studies.

	Abdelhalim (2014)		Elhendawy (2015)		Ghobrial (2018)		Al-Wahab (2019)	
	EBL	APC	EBL	APC	EBL	APC	EBL	APC
No. of patients	20	20	44	44	20	20	18	18
Mean age (years)	55.65	57.17	51.41	53.09	9.65	7.8	65	60
Bleeding (occult/overt [%])	20/80	45/55	NI	NI	0/100	0/100	17/83	23/77
Gender (male/female)	9/11	10/10	19/25	15/29	13/7	11/9	11/7	15/3
APC settings	–	60 W 2 L/min	–	60 W 2 L/min	–	40 W 1 L/min	–	50 W 2 L/min
No. of bands	Up to 18 bands	–	Up to 12 bands	–	Up to 6 bands	–	NI	–
No. of sessions required	2. 25±0.64	5.5±3.76	2.93±0.846	3.48±0.902	1.85±0.18	4.15±1.22	2.25±0.38	2.5±0.57
Procedure time (min)	NI	NI	NI	NI	9.4±1.21	15.37±1.56	NI	NI
Recurrence of bleeding	1/20	7/20	NI	NI	1/20	7/20	3/18	8/18
Endoscopic eradication	19/20	12/20	NI	NI	19/20	12/20	13/18	10/18
Hb level after intervention	9.68±1.31	8.92±2.12	10.31±1.01	9.85± 0.906	9.2±0.84	9.02±1.32	8.8±1	8.7±0.9
Mean no. of hospitalizations	0.05±0.22	0.5±0.95	NI	NI	0.67±0.15	0.95±0.18	NI	NI
Transfusion requirements (units)	0.15±0.67	2.00±2.97	2.5±0.70	4.6±0.89	0.44±0.1	1.0±0.67	NI	NI
Transfusion requirements (no. of patients, %)	NI	NI	2 (4.5)	5 (11.4)	NI	NI	3 (17)	7 (39)
Adverse events (no. of patients, %)	0/20 (0)	0/20 (0)	6/44 (13)	9/44 (20)	14/20 (70)	2/20 (10)	6/18 (33)	0/18 (0)

NI: not informed; W: watts; L: liters.

Transfusion requirement

A total of 116 patients were analyzed from three studies[21-23]. The EBL group required fewer transfusions (mean difference [MD]: 1.49, 95%CI: [0.28, 2.71], p=0.02, I^2=96%) (Annex Figure 4). The GRADE analysis revealed a very low certainty of evidence.

Number of sessions

A total of 204 patients were analyzed from four studies[21-24]. The number of sessions required for complete obliteration of the lesions was higher in the APC group (MD: 1.38, 95%CI: [0.35, 2.42], p=0.009, I^2=94%) (Annex Figure 5). The GRADE analysis revealed a very low certainty of evidence.

Mean number of hospitalizations

A total of 80 patients were analyzed from two studies[21,23]. The EBL group required fewer hospitalizations than patients in the CPA group (MD: 0.29, 95%CI [0.19, 0.39], p<0.00001, I^2=0%) (Annex Figure 6). The GRADE analysis revealed a moderate quality of evidence.

Adverse events

A total of 204 patients were analyzed from four studies[21-24]. There was no difference between the two techniques (RD: –0.20, 95%CI: [–0.48, 0.07], p=0.15, I^2=91%) (Annex Figure 7). The GRADE analysis revealed a very low certainty of evidence.

DISCUSSION

Gastric antral vascular ectasia is a common entity that can be present in both patients with and without cirrhosis and that has a different spectrum of treatment and behavior than PHG. It carries significant morbidity and financial impact when patients are often hospitalized, requiring endoscopic procedures and blood transfusions. Nonetheless, few high-quality studies have evaluated the most optimal treatment modality. We performed the first systematic review and meta-analysis, including only RCTs and evaluating both APC and EBL in the treatment of this entity (level of evidence 1A).

This meta-analysis demonstrated that EBL has higher rates of endoscopic eradication, a less recurrence of bleeding, and a reduction in transfusion requirements. These results may be explained because EBL acts in deeper gastric wall layers, leading to thrombosis and ischemia of the mucosa and submucosa, which are subsequently replaced by fibrous tissue (Figure 1). Consequently, blood flow in the feeding vessels of GAVE is interrupted and then GAVE is eradicated. In contrast, APC acts only on the mucosa. Since GAVE is characterized by dilation of the mucosal and submucosal vessels with focal thrombosis, it is understandable that the deeper action of EBL will promote less recurrence of these lesions.

Regarding the number of hospitalizations, we included the meta-analysis data from two studies, reporting a significant reduction in the mean number of hospitalizations per patient in the EBL group. Concerning safety, only a few adverse events

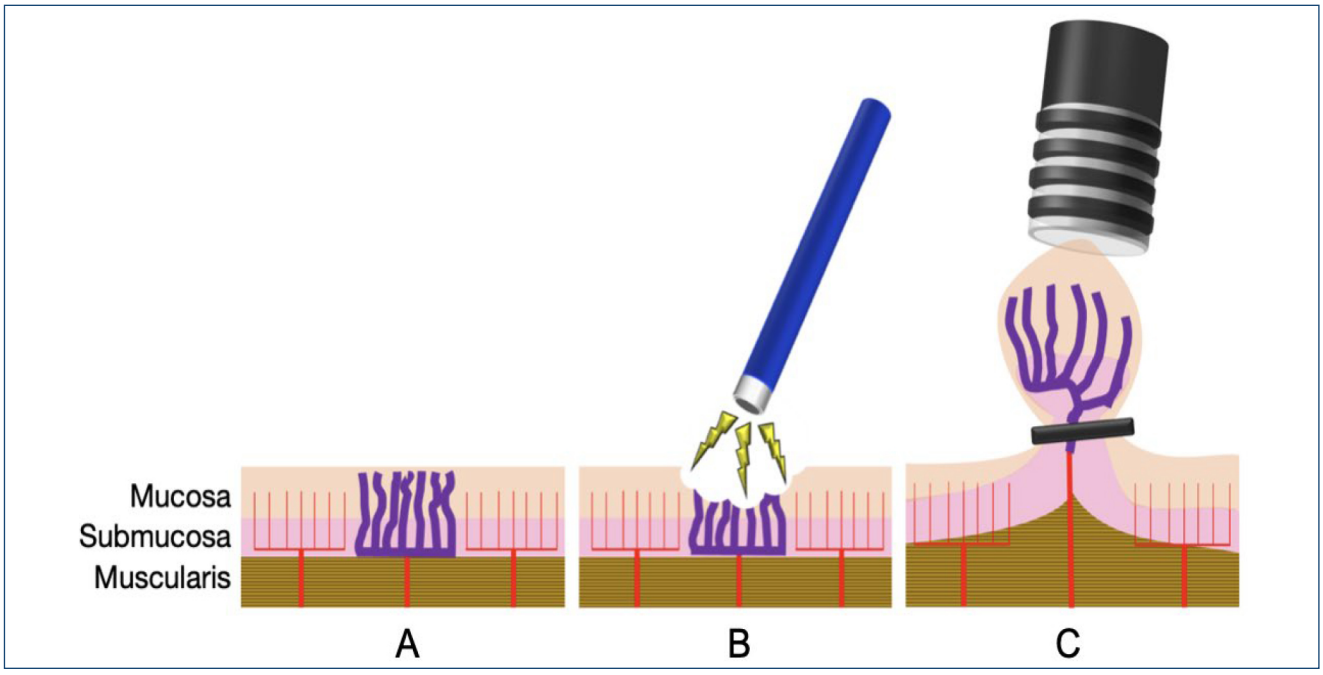

Figure 1. A: Mucosa with gastric antral vascular ectasia. B: Treatment with argon plasma coagulation. C: Treatment with endoscopic band ligation.

were reported, such as fever, epigastric pain, abdominal distension, mild bleeding, and vomiting, with no difference within the procedures. No serious adverse events were observed, proving the safety of both methods according to the recommendations of the American Society for Gastrointestinal Endoscopy Quality Task Force[25]. One of the included studies[24] reported the development of hyperplastic polyps in some patients undergoing EBL. However, an uncommon complication has been reported with APC and RFA[26,27].

Despite the efficiency of both techniques to treat GAVE, EBL still has more advantages. It is more widely available and less time-consuming, and since it requires fewer endoscopic sessions, it is probably associated with reduced costs. However, in some situations, both techniques can be performed complementarily, especially in fibrotic areas related to the previous banding that are difficult to suction and to achieve band deployment.

The exposed results are consistent in patients with chronic liver disease, which represent the etiology of a considerable amount of the GAVE cases encountered in daily practice. However, other conditions associated with GAVE, such as connective tissue disorders, end-stage renal disease, and bone marrow transplantation, were not evaluated in this study[9]. Since the pathogenesis is not fully understood, it is not possible to affirm that our results may be extrapolated to other diseases. Nevertheless, there are two retrospective studies, including patients with noncirrhotic GAVE and also reporting the superiority of EBL over APC.

In this study, we analyzed the two main endoscopic options (i.e., EBL and APC) for the treatment of GAVE. Promising emergent techniques, such as radiofrequency and hybrid-APC, have not been evaluated in RCTs and, therefore, not included in our study. More studies are needed to compare APC and EBL techniques. The treatment of GAVE is also limited by an incomplete understanding of its pathogenesis. Hence, it is possible that a more accurate understanding of the pathophysiology can lead to a better management of this condition.

Our study has several limitations. Few articles were included in the analysis. However, this is a limitation due to the small number of RCT published on this subject. All the included studies were conducted in the same country (Egypt), which is ranked among countries having the highest death rates of cirrhosis[30]. Nonetheless, our results were consistent with previous observational studies that were conducted in other countries as well[28,29,31]. Not all outcomes were evaluated in every trial. Significant heterogeneity was identified among the studies, which might be explained by the following reasons: the severity of the liver disease varies among the studies and different APC settings alongside a variable number of endoscopic bands applied on each session. These aforementioned reasons might have influenced the outcomes. One of the included studies was performed in children with liver disease[23]. However, the results were consistent with the other studies, probably due to the same pathophysiology in patients with chronic liver disease. Regarding the transfusion requirements, none of the studies informed the cutoff value to indicate blood transfusions. Nevertheless, all individual studies consistently reported fewer transfusions in patients with EBL.

CONCLUSIONS

We performed an extensive systematic and in-depth critical evaluation of the best level of evidence on this subject. The available data recommended EBL as the first option for the treatment of GAVE. We strongly believe that this can significantly and positively impact the care and management of patients suffering from this condition.

Summary of recommendations

EBL and APC are effective and safe procedures for the endoscopic treatment of GAVE. EBL is superior to APC in terms of endoscopic eradication rates, recurrence of bleeding, and need for transfusion. In some situations, both techniques can be performed in a complementary way. Both interventions had similar adverse events.

The level of evidence varies from high to very low, depending on the outcome analyzed.

AUTHORS' CONTRIBUTIONS

BSH, IBR, EGHM: Conceptualization. **BSH:** Data curation. **BSH, IBR, MPF, VMS, FCM, GHPO, WMB, EGHM:** Formal Analysis. **BSH, WBM, EGHM:** Methodology. **BSH, WBM:** Investigation, Project administration, Software. **WBM, EGHM:** Resources, Supervision, Validation. **BSH, IBR, WBM, EGHM:** Visualization. **BSH, IBR, MPF, VMS, EGHM:** Writing – original draft. **BSH, IBR, MPF, VMS, FCM, GHPO, EGHM:** Writing – review & editing.

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ANNEX

METHODS

Protocol and Registration

This study was performed in conformity with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, and it was registered in the International Prospective Register of Systematic Reviews under the file number CRD42020191896. This study was approved by the Ethics Committee of Hospital das Clínicas, Faculty of Medicine at The University of São Paulo.

Eligibility criteria

We screened all studies with the following inclusion criteria:

a. Study design: only RCTs.
b. Population: patients with GAVE, irrespective of age.
c. Type of intervention: argon plasma coagulation and EBL.
d. Outcomes: endoscopic eradication, recurrence of bleeding, blood transfusion requirements, number of sessions needed, number of hospitalizations, and adverse events.

Search and Study Selection

We performed a search in electronic databases (i.e., MEDLINE, Embase, Cochrane, and LILACS) and grey literature from their inception to March 2021. Only RCTs comparing APC and EBL for the treatment of GAVE were included. No restrictions were set for publication date or language. The search strategy in MEDLINE was gastric antral vascular ectasia OR antral vascular ectasia OR antral vascular ectasia OR watermelon stomach OR watermelon stomachs. In the other databases, the search was performed with the term "gastric antral vascular ectasia." Two independent investigators conducted the screening for eligibility. Any disagreements were resolved by consultation with a third reviewer.

Data collection process

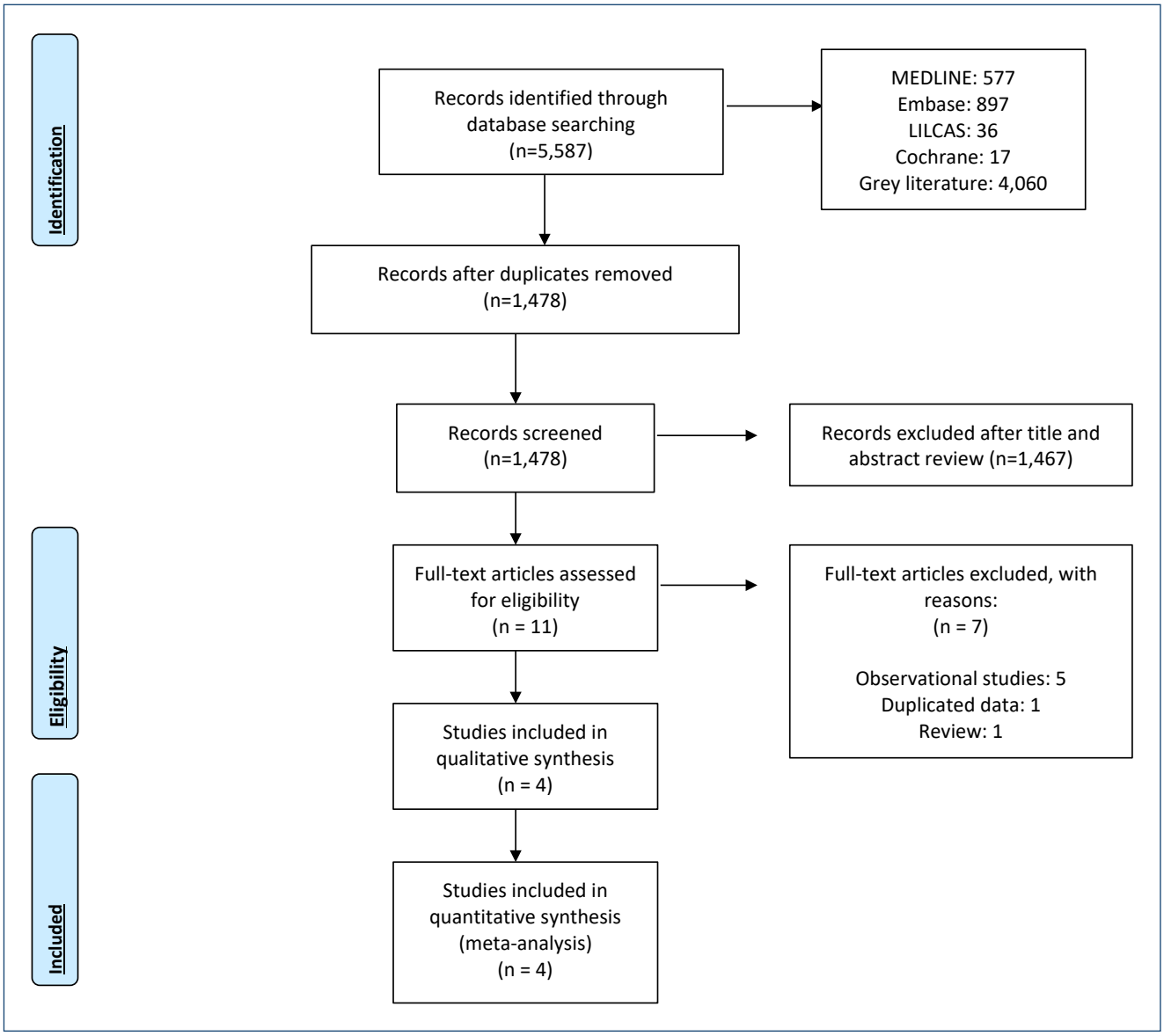
The following data were extracted: name and year of the study, number of patients, age, gender, type of bleeding (i.e., occult or overt), argon plasma settings, number of bands, Child-Pugh score, number of sessions, procedure time, recurrence of bleeding, endoscopic eradication, hemoglobin level after the intervention, number of hospitalizations, transfusion requirements, and adverse events. When data of the published articles were insufficient, the corresponding authors were consulted by e-mail for further elucidation.

Risk of bias and quality of studies

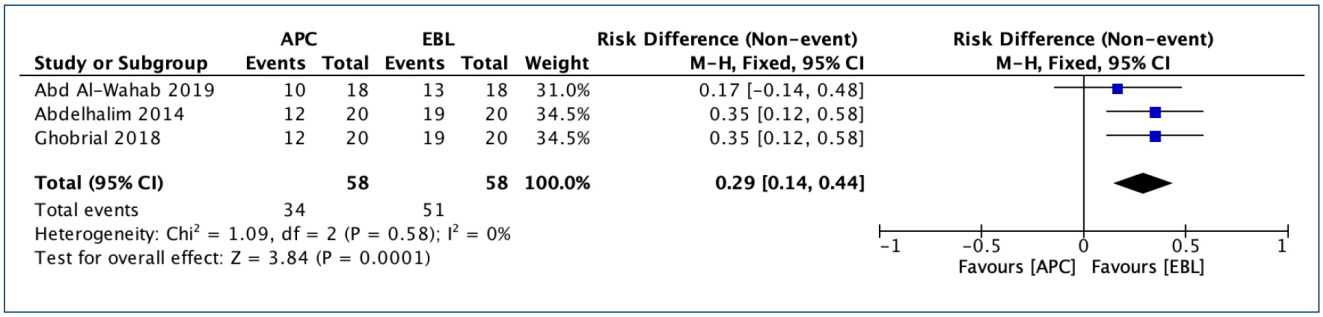
The risk of bias was assessed by the RoB2. We analyzed the risk of bias for each outcome of every included study. The quality of the evidence was assessed using the GRADE criteria with the GRADEpro Guideline Development Tool software.

Data synthesis and statistical analysis

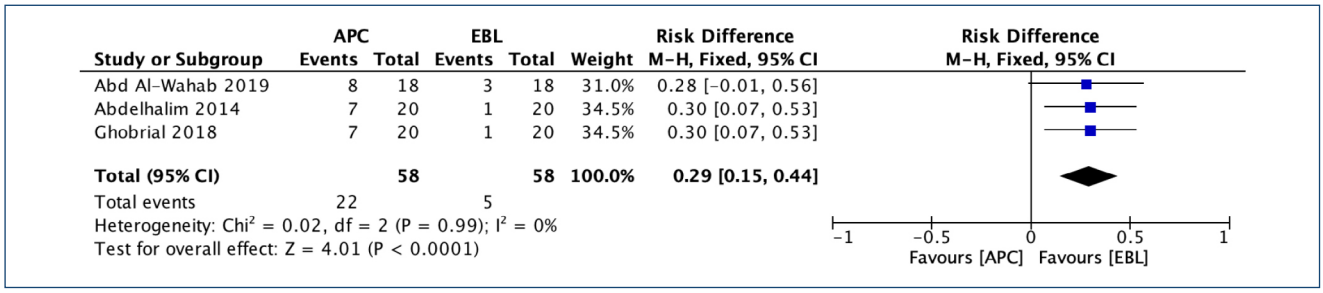
The sample mean was estimated, when needed, from its median and range[31]. The statistical analyses were conducted using the RevMan software, version 5.4. The Mantel–Haenszel test was used for categorical variables, and inverse variance was used for continuous variables. Heterogeneity was evaluated using the Higgins test (I^2). I^2 values higher than 50% were considered substantial heterogeneity[30]. We used the fixed effect when $I^2 < 50\%$. If $I^2 > 50\%$, we performed a sensitivity analysis through a funnel plot to identify possible outliers. If the sample became homogeneous after the exclusion, the studies were permanently excluded (considered true outliers), and the fixed model was used. When there was no outlier or heterogeneity remained high after the outliers were excluded, we used the random effect to reduce the impact of heterogeneity on the final result. Outcome measures were described as mean difference or risk difference, with their corresponding 95%CIs. Intention-to-treat analysis was performed in all studies.



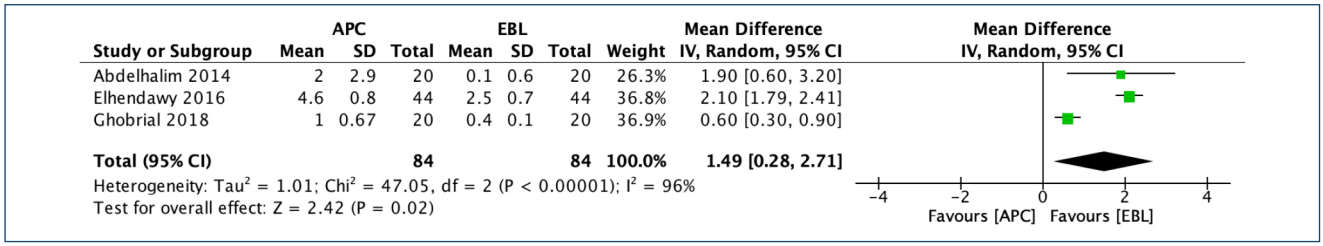
Annex Figure 1. PRISMA flow diagram.



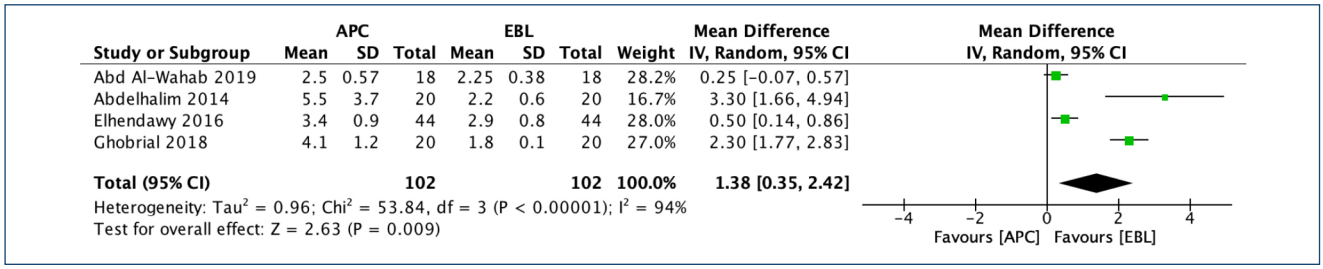
Annex Figure 2. Endoscopic eradication.



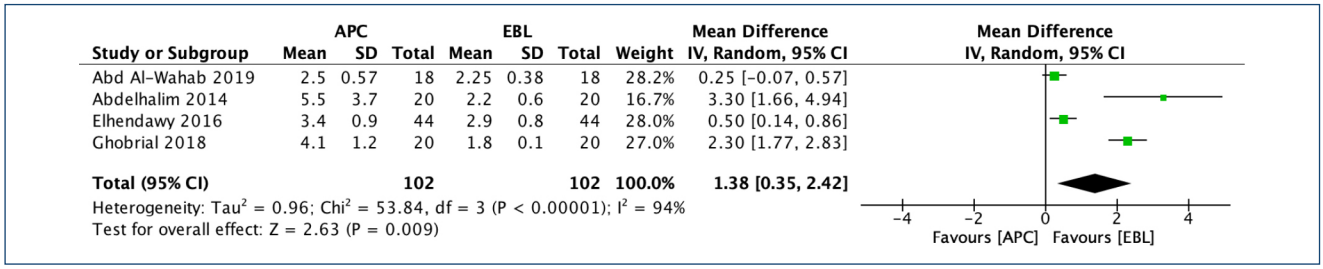
Annex Figure 3. Recurrence of bleeding.



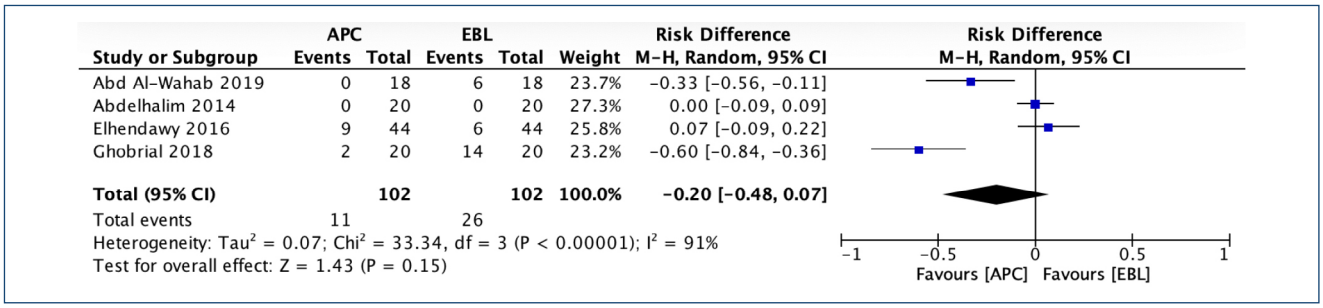
Annex Figure 4. Transfusion requirement.



Annex Figure 5. Number of sessions.



Annex Figure 6. Number of hospitalizations.



Annex Figure 7. Adverse events.

Annex Table 1. Risk of bias (RoB-2) tool.

Study		Elhendawy	Ghobrial	Abdelhalim	Abd Al-Wahab
DOMAIN 1 RANDOMIZATION PROCESS	1.1 Was the allocation sequence random?	Y	Y	Y	Y
	1.2 Allocation sequence concealed?	Y	Y	PY	Y
	1.3 Baseline imbalances suggest a problem with the randomization process?	N	N	N	N
	Risk of bias judgment	LOW	LOW	LOW	LOW
DOMAIN 2 DEVIATIONS FROM INTENDED INTERVENTIONS	2.1. Participants aware of their assigned intervention?	PY	PY	PY	PY
	2.2. Carers and people delivering the interventions aware of participants' assigned intervention?	Y	Y	Y	Y
	2.3. Were there deviations that arose because of the trial context?	N	N	N	N
	2.6 Appropriate analysis to estimate the effect of assignment to intervention?	PY	PY	PY	PY
	Risk-of-bias judgment	LOW	LOW	LOW	LOW
DOMAIN 3 MISSING OUTCOME DATA	3.1 Were data for this outcome available for all, or nearly all, randomized participants?	PY	Y	PY	PY
	Risk-of-bias judgment	LOW	LOW	LOW	LOW
DOMAIN 4 MEASUREMENT OF THE OUTCOME	4.1 Was the method of measuring the outcome inappropriate?	N	N	N	N
	4.2 Measurement or ascertainment of outcome differs between groups?	N	N	N	N
	4.3 Outcome assessors aware of the intervention received by participants?	Y	Y	Y	Y
	4.4 Could assessment of the outcome have been influenced by knowledge of intervention received?	PN	PN	PN	PN
	Risk-of-bias judgment	LOW	LOW	LOW	LOW
DOMAIN 5 SELECTION OF THE REPORTED RESULT	5.1 Trial analyzed in accordance with a prespecified analysis plan?	PY	PY	PY	PY
	5.2 Is the result selected from multiple eligible outcome measurements?	PN	PN	PN	PN
	5.3 Is the result selected from multiple eligible analyses of the data?	PN	PN	PN	PN
	Risk-of-bias judgment	LOW	LOW	LOW	LOW
OVERALL RISK OF BIAS		LOW	LOW	LOW	LOW

Y: yes; PY: probably yes; N: no; PN: probably no.

Annex Table 2. GRADE.

Certainty assessment							N° of patients		Effect		Certainty	Importance
N° of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	EBL	APC	Relative (95% CI)	Absolute (95% CI)		
Number of sessions												
4	Randomized trials	Not serious	Very serious[a]	Not serious	Not serious	Publication bias strongly suspected[b]	102	102	–	MD **1.38 higher** (0.35 higher to 2.42 higher)	⊕○○○ Very low	
Mean number of hospitalizations												
2	Randomized trials	Not serious	Not serious	Serious[c]	Not serious	None	40	40	–	MD **0.29 higher** (0.19 higher to 0.39 higher)	⊕⊕⊕○ Moderate	
Recurrence of bleeding												
3	Randomized trials	Not serious	Not serious	Not serious	Not serious	None	22/58 (37.9%)	5/58 (8.6%)	RR 4.40 (1.80–10.77)	**293 more per 1,000** (from 69 more to 842 more)	⊕⊕⊕⊕ High	
Endoscopic eradication												
3	Randomized trials	Not serious	Not serious	Not serious	Not serious	None	34/58 (58.6%)	51/58 (87.9%)	RR 3.43 (1.61–7.30)	**1,000 more per 1,000** (from 536 more to 1,000 more)	⊕⊕⊕⊕ High	
Blood transfusion (units)												
3	Randomized trials	Not serious	Very serious[a]	Not serious	Not serious	Publication bias strongly suspected[b]	84	84	–	MD **1.49 higher** (0.28 higher to 2.71 higher)	⊕○○○ Very low	
Adverse events												
4	Randomized trials	Not serious	Very serious[a]	Not serious	Serious[d]	Publication bias strongly suspected[b]	11/102 (10.8%)	26/102 (25.5%)	RR 0.31 (0.04–2.38)	**176 fewer per 1,000** (from 245 fewer to 352 more)	⊕○○○ Very low	

CI: confidence interval; **MD:** mean difference; **RR:** risk ratio.

[a] High levels of heterogeneity. [b] Presence of outliers. [c] Surrogate endpoint. [d] Wide confidence interval range.

Source: Endoscopic band ligation versus argon plasma coagulation in the treatment of gastric antral vascular ectasia: a Systematic Review and Meta-analysis of Randomized Controlled Trials. Cochrane Database of Systematic Reviews [Year], Issue [Issue].

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COVID-19 in HIV-infected individuals

Pathum Sookaromdee[1*], Viroj Wiwanitkit[2]

Dear Editor,

We would like to share ideas on the publication entitled "Potential impact of the COVID-19 in HIV-infected individuals: a systematic review"[1]. de Medeiros et al.[1] noted that "HIV did not show any relevance directly with the occurrence of COVID-19. Some studies suggest … to control HIV infection could be used to prevent COVID-19 infection"[1]. In fact, the clinical association between COVID-19 and HIV infection is interesting. According to the pathobiological process of both infections, there is no common pathway regarding receptor and target cell. Hence, there should be no direct clinical association. Regarding the effect of antiviral therapy, the common antiviral activity of some antiviral drugs might be useful for the management of COVID-19. However, the dose adjustment might be required[2]. Nevertheless, the common clinical and geographical epidemiology of these two diseases should be discussed. In many developing countries, social determinants of health of HIV cases are usually poor and might support getting COVID-19[3].

AUTHORS' CONTRIBUTIONS

PS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing. **VW:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

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Comment on "Hyperglycemia in pregnancy: sleep alterations, comorbidities, and pharmacotherapy"

Yushun You[1], Zhipeng Li[1], Xiaohong Liu[1*]

Dear Editor,

We were happy to read the article written by Facanha et al[1]. The study found that sleep problems in pregnant women with diabetes are very common, which might be type 1 diabetes (T1DM) and type 2 diabetes (T2DM) or gestational diabetes mellitus (GDM). Another finding of the study was metformin, a commonly used oral hypoglycemic drug, and higher parity were independently associated with poor sleep quality in GDM patients. Although their findings point to some problems and offer innovative ideas, we believe some issues should be further discussed.

The purpose of this study has been detailed at the end of the introduction, that is, to examine the sleep quality and sleep duration of patients with hyperglycemia secondary to T1DM, T2DM, and GDM in the middle and late stages of pregnancy and to analyze the factors that may affect sleep status. However, in our opinion, the title of this article may have been enlarged. To make the title of this article more accurate and reasonable, we suggest changing the title of this article from "Hyperglycemia in pregnancy: sleep alterations, comorbidities, and pharmacotherapy" to "Mid-late pregnant women: The effect of blood glucose comorbidity on sleep and drug treatment."

We found another article[2] pointing out that the Pittsburgh Sleep Quality Index (PSQI) cutoff score of 5 is more suitable for expectant mothers, but may not be suitable for pregnant women, especially in the late pregnancy. Therefore, we suggest that different scoring criteria should be selected for different gestation periods, or the PSQI score level should be appropriately increased.

In the Methods section, the authors explained the data sources provided by the Center for Diabetes and Hypertension (CIDH-CE) and conducted a cross-sectional study based on this. However, cross-sectional studies have inherent limitations and are prone to various biases, such as no response bias, recall bias or reporting bias, and measurement bias. In addition, the samples of the cross-sectional study should be obtained through random sampling instead of non-random sampling, so the authenticity of the conclusions of this study remains to be verified. If the authors or other investigators need to confirm the results of this study, we recommend expanding the sample size, improving the sleep score criteria, and random sampling to ensure sample quality.

AUTHORS' CONTRIBUTION

YY: Formal Analysis, Writing – original draft. **ZL:** Writing – review & editing. **XL:** Conceptualization, Writing – review & editing.

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Comment on "Nutritional aspects and cardiovascular risk in systemic lupus erythematosus"

Roujun Pan[1], Zhipeng Li[1], Lianping He[1*]

Dear Editor,

We were very happy to read the valuable article written by Calzza et al.[1] This article discovered the relationship between systemic lupus erythematosus (SLE), cardiovascular disease (CVD), and metabolic syndrome (MS). Patients suffering from SLE have a significantly increased risk of CVD and MS. Although the author's findings have been proven by good practice and supported by data, we believe that there are still some problems that can be further explored.

First of all, in the method part of the summary, we suggest supplementing the statistical methods used in this article, such as the use of Pearson's chi-square test to perform probability analysis on the categorical data of unpaired samples.

In addition, it is well known that the prevalence of SLE is significantly higher in women than in men. The primary factor for this is suspected to be the difference in sex hormone levels. SLE patients are usually accompanied by fluctuations in sex hormone levels, and sex hormone levels also have a greater impact on body weight[2]. For example, Leeners et al.[3] pointed out that estrogen plays a leading role in the causes and consequences of female obesity. At the same time, estrogen also has a myocardial protective effect, which can reduce the risk of CVD to a certain extent. The sample of women in this study has a large age span, including both 25–49-year-old women of normal childbearing age and menopausal women more than 50 years old. The levels of female sex hormones are not the same in different periods, especially after menopause, estrogen levels will be significantly reduced. We propose to stratify the samples by age to investigate the risk of SLE with MS and CVD at different ages.

Finally, we believe that the representativeness of the samples in this study is relatively weak. The reason is that the Systemic Lupus International Collaborating Clinics Damage Index (SLICC) and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores are low, and it is suggested that SLE has a relatively mild disease course and relatively weak influence on the host function and metabolism. Therefore, we recommend increasing the SLICC and SLEDAI scores as much as possible. In addition, the sample content in this experiment is not well-balanced, and the proportion of women is as high as 90%. This is unfavorable for the gender-controlled analysis of the experimental results. We suggest expanding the sample content as much as possible to provide a more reliable reference for the experiment.

AUTHORS' CONTRIBUTIONS

RP: Formal Analysis, Writing – original draft. **ZL**: Writing – review & editing. **LH:** Conceptualization, Writing – review & editing.

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Comment on "Stiffness degree of ankle range of motion in diabetic patients with atypical amputation"

Minjie Jiang[1], Chenchen Pan[1], Lianping He[1*]

Dear Editor,

We are more than honored to read the high-level articles published by Simón-Pérez et al.[1] Their study found that feet with atypical distal amputations feature the classification of dorsal flexing movement in the ankle lower than the contralateral foot. However, as far as I am concerned, there are still some issues worth discussing with the author.

First, the author did not explain what statistical methods were used in the method section. We do not know what the p-value is which indicates that the result is significant. In addition, both type 1 and type 2 diabetes were included in the sample. We think that author should further discuss in-depth the effects of two different types of diabetes on the stiffness of ankle joints. And the sample size of the patients with diabetes 1 was too small.

Second, the author collected the general demographic characteristics of the sample in the study. This is worth approving. However, the author should then analyze the relativity between age, gender, weight, size, and body mass index (BMI) and stiffness degree of ankle joint activity in diabetic patients. In contrast, the author stated that they collected the general demographic characteristic information of size. But we do not find the corresponding information in Table 1. Besides, the sample size of women patients was too small.

The author pointed out in the discussion section that age, smoking, BMI, and the level of Hb1Ac did not affect the classification of ankle stiffness degree. We are confused about how they came to this conclusion. The author did not make relevant statistical analysis in this article, and we cannot even see the corresponding p-value.

Finally, in the study, the conclusion is also confusing, which seems to be contrary to the research goal of this article. Foot Posture Index (FPI) was a specific value rather than a tendency. The author stated that FPI reflects the stiffness degree of ankle joint in the patients with diabetes.

AUTHORS' CONTRIBUTIONS

MJ: formal analysis, writing – original draft, and data curation. **CP**: conceptualization and writing – review and editing. **LH**: Conceptualization, investigation, funding acquisition, methodology, project administration, resources software, supervision, validation, visualization, writing – review and editing.

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SHORT COMMUNICATION

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Autoimmunity overlaps primary biliary cirrhosis: a not straightforward diagnosis

Tiago Isidoro Duarte[1*], Laura Moreira[2], César Burgi Vieira[1], Nuno Germano[1]

During the last decades, the diagnostic and clinical approach of acute liver failure has been improved due to a progressive understanding of the disease and better outcomes[1]. The most frequent etiologies are viral hepatitis and acetaminophen intoxication, the former in developing countries and the latter in Europe and the United States. Nevertheless, vascular phenomena, infiltrative diseases, or even autoimmune disorders must also be considered[2]. Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder usually associated with malar rash, arthralgias, cytopenia, serositis, renal failure, endocarditis, and antiphospholipid syndrome[3,4]. Liver involvement is normally not a part of the spectrum but observed in up to 60% of SLE patients. Moreover, the co-occurrence of autoimmune hepatitis (AIH) and SLE is considered to be rare, and only few reports have been published so far. In this study, we presented a case report of a patient with this overlap syndrome.

A 57-year-old women of Guinean origin presented to the Emergency Department with a 7-day history of jaundice and pruritus. She had a history of arterial hypertension, dyslipidemia, and obesity, treated with lisinopril and pravastatin. She had traveled to a rural area of Guinea-Bissau before 3 months. There was no history of alcohol, drugs, or consumption of natural products. Remarkable physical examination findings included lethargy and flapping without fever. She also had pruritic and papulovesicular skin lesions on torso, upper limbs, and palms but no scalp achievement. Vital signs were normal, and abdominal examination did not reveal pain, tenderness, hepatosplenomegaly, ascites, or lymphadenopathy. Blood tests revealed anemia (hemoglobin 10 g/dL) and thrombocytopenia (platelet account 112×10^9/L), and erythrocyte sedimentation rate of 81 mm/h. Liver tests showed a cytocholestatic pattern, with a total bilirubin of 20 mg/dL, direct bilirubin of 12 mg/dL, aspartate aminotransferase of 900 IU/L, alanine aminotransferase of 300 IU/L, alkaline phosphatase of 300 IU/L, ammonia of 130 µmol/L, hypoalbuminemia of 20.9 g/L, international normalized ratio of 3:1, fibrinogen of 1.04 g/L, and V factor of 39%. Arterial blood gas revealed an elevation in lactate level (3.0 mmol/L). Acetaminophen levels were negative. Abdominal ultrasound demonstrated a heterogeneous liver with fatty infiltration but without focal lesions or abnormal vascular patterns. Unfortunately, no skin biopsies were performed.

She was admitted to the intensive care department with the diagnosis of acute/subacute liver failure. An extensive workup diagnosis was performed: viral hepatitis A, B, C, and E and herpes simplex serologies were negative. Ceruloplasmin was normal. *Coxiella burnetii*, rickettsia, brucellosis, schistosomiasis, leptospirosis, and toxoplasmosis were also negative. Polymerase chain reaction (PCR) for Cytomegalovirus (CMV) was positive with viral load of 140 IU/mL and immunoglobulin G (IgG) antibodies for the varicella-zoster virus were also positive, so treatment with valganciclovir was initiated. *N*-acetylcysteine protocol was maintained for 5 days and lactulose therapy was performed. According to complementary results, serologies were positive for antinuclear antibodies (homogeneous pattern, 1:640), anti-dsDNA (773 IU/mL), anti-nucleosome antibodies (>20,000 U/mL), ENA Ro52KD 3+, and hypergammaglobulinemia (IgG+). Serologies were negative for anti-smooth muscle antibody, anti-mitochondrial (AMA), anti-liver kidney microsomal type 1 (LKM 1), anti-cardiolipin, anti-Scl70, and anti-Jo1. C3 and C4 were negative. Transjugular liver biopsy revealed extensive necrosis, portal infiltration by neutrophils, cholestasis, and giant multinuclear cells without steatosis (Figures 1–3). CMV or Epstein-Barr virus (EBV) was not identified by immunohistochemistry. Based on laboratory tests and serologies, a diagnosis of an autoimmune cause was made. Nevertheless, she evolved with bacteremia to *Escherichia coli* and worsening encephalopathy, so immunosuppressive therapy was postponed, and she underwent liver transplantation at day 7. Postoperative period evolved well with

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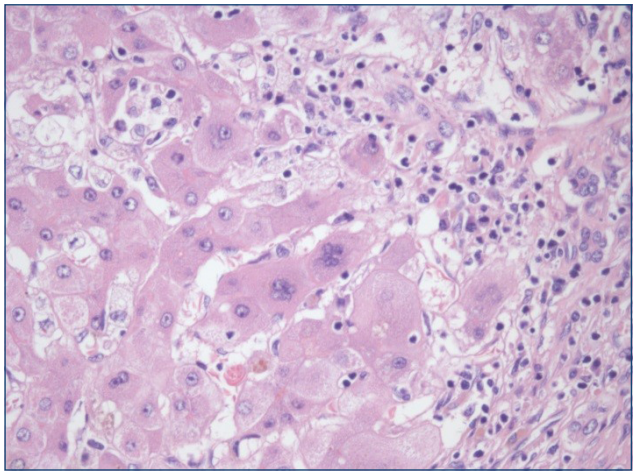

Figure 1. Multinucleated hepatocytes.

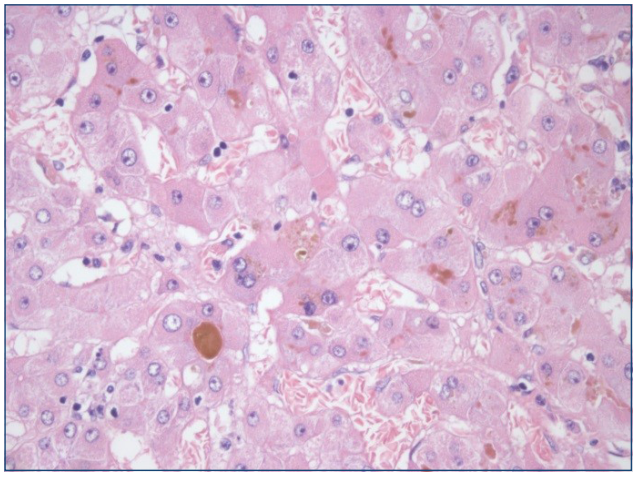

Figure 2. Canalicular and parenchymal cholestasis.

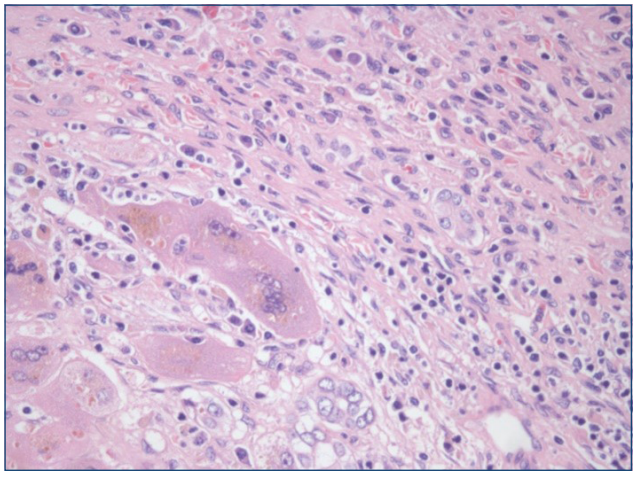

Figure 3. Multinucleated hepatocytes.

self-limited cholestasis. Abdominal ultrasound with Doppler was normal. Repeated PCR for CMV was negative. She was discharged after 28 days post-transplant surgery. Immunosuppression therapy was performed first with methylprednisolone and basiliximab and then switched to prednisolone and tacrolimus.

After 3 months, she was again admitted with a progressive liver cytolysis and cholestatic pattern. Abdominal computed tomography revealed a biliary stenosis, and she was then treated with a new biliodigestive derivation. She evolved to hepatic artery thrombosis and subsequent ischemic liver failure and again underwent liver transplantation. Once again, during the postoperative period, she presented a progressive cholestasis under immunosuppression therapy with prednisolone and tacrolimus. Abdominal computed tomography scan was normal. Mycophenolate mofetil was started with progressive regression. Results of 3-month follow-up laboratory liver tests were normal.

SLE is a multisystem autoimmune disorder in which liver involvement is normally not common[4]. AIH, also known as lupoid hepatitis, is an autoimmune liver disease caused by the presence of antibodies (antinuclear antibodies) and hypergammaglobulinemia. The existence of overlap syndromes linking SLE with other autoimmune liver diseases is a matter of controversies, and data in the literature are scarce[5]. Primary biliary cirrhosis (PBC) is a chronic cholestatic disease estimated to evolve to liver failure in 15% of cases[6]. Besides, AMA antibodies are positive in 95% of patients with PBC, and clinical presentation, liver histology, and natural history in patients with PBC without AMA antibodies are nearly identical. The study patients are in line with literature as liver biopsy demonstrates the typical features of bile duct destruction and granulomas[6].

The difference between the hepatic involvement in SLE and AIH has not been clearly defined due to similarities in the clinical and biochemical features[7]. In our report, an unusual presentation associated with a fulminant liver failure course made the diagnostic a challenging task, delaying immunosuppression treatment initiation. A PBC/AIH overlap syndrome may also refer to patients with sequential PBC followed by AIH; less commonly, AIH followed by PBC has been described[6].

Furthermore, despite liver transplantation, she presented later with new liver cytolysis and cholestatic pattern without any biliary abnormalities under immunosuppression therapy with prednisolone and tacrolimus. According to literature findings, some patients with lupus hepatitis may be refractory not only to corticosteroids but also to conventional immunosuppressants, such as cyclophosphamide, tacrolimus, and azathioprine[8]. As in our case, mycophenolate mofetil led to a rapid resolution of liver test abnormalities.

In conclusion, AIH and SLE are distinct diseases, in which a combination of clinical symptoms and diagnostics markers overlaps. In our view, AIH needs to be considered in the differential diagnosis of any SLE patient with elevated liver enzymes. Early referencing to a Liver Transplant Centre plays an important key role in these patients' approach and treatment.

ETHICAL APPROVAL

Our institution does not require ethical approval for reporting individual cases. The patient described herein had given consent to the use of de-identified patient data for use in research. Written informed consent was obtained from the patient for this anonymized information to be published in this article.

AUTHORS' CONTRIBUTIONS

TID: conceptualization, formal analysis, writing – original draft, and writing – review and editing. **LM**: formal analysis and writing – original draft. **CBV**: writing – original draft and writing – review and editing. **NG**: writing – review and editing.

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ORIGINAL ARTICLE

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Secondary bacterial infections in patients with coronavirus disease 2019-associated pneumonia

Ozlem Aydemir[1], Yusuf Aydemir[2*], Elif Özözen Şahin[1], Fatih Şahin[3], Mehmet Koroglu[1], Ali Fuat Erdem[3]

SUMMARY

OBJECTIVE: The vast majority of patients who hospitalized with coronavirus disease 2019 are given empirical antibiotic therapy. However, information on the frequency, microorganism species, and resistance rates of secondary bacterial infections in coronavirus disease 2019 patients are insufficient. We aimed to show the frequency of secondary infections and resistance conditions in patients with coronavirus disease 2019 hospitalized in the intensive care unit.

METHODS: The results of tracheal aspirate culture, blood culture, and urine culture obtained from coronavirus disease 2019 patients – at least 2 days after their admission to the intensive care unit – were examined microbiologically.

RESULTS: A total of 514 patients hospitalized in intensive care unit were included in our study. Tracheal aspirate, blood, or urine cultures were collected from 369 patients (71.8%). Bacterial reproduction was detected in at least one sample in 171 (33.3%) of all patients. The rate of respiratory tract infection and/or bloodstream infection was found to be 21%. *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* in tracheal aspirate culture; *Coagulase-negative staphylococci*, *K. pneumoniae*, and *A. baumannii* in blood culture; and *Escherichia coli*, *K. pneumoniae*, and *Enterococcus faecalis* in urine culture were the most common microorganisms. *A. baumannii* was resistant to most antibiotics except colistin and *P. aeruginosa* strains were resistant to most antibiotics except amikacin, colistin, cefepime, and imipenem. In *K. pneumoniae*, the highest meropenem sensitivity (73%) was observed; there was a strong resistance to most of the remaining antibiotics.

CONCLUSIONS: We think that our study can be useful in choosing empirical antibiotic therapy in the coronavirus disease 2019 pandemic and reducing the mortality that may occur with secondary infection.

KEYWORDS: COVID-19. Secondary infection. Nasocomial infections. Antimicrobial Susceptibility.

INTRODUCTION

Among patients with coronavirus disease 2019 (COVID-19), 5–15% have moderate or severe symptoms and require hospitalization, and some require intensive care unit (ICU) follow-up. Prolonged ICU stays and use of immunosuppressor treatment regimens, such as steroids and interleukin inhibitors, may increase the frequency of secondary bacterial infections (SBIs) in COVID-19 patients[1]. SBI occurs in many COVID-19 patients and is associated with worse outcomes, including death[2].

SBIs have become the hidden threat behind COVID-19[3]. Although most patients with COVID-19 are given empirical antibiotic therapy, there is no enough information about the type of bacterial agents that develop in these patients and their antibiotic susceptibility[4]. Therefore, data obtained from other viral pneumonia treatments are considered when choosing the antibiotics. During the pandemic period, different antimicrobial treatment protocols were recommended and, accordingly, many broad-spectrum antibacterial agents were used in empirical treatment before SBIs were confirmed in the majority of patients. We are using more antibiotics in our fight to save COVID-19 patients from bacterial coinfections, and it is important to consider how this could affect the prevalence of antibiotic-resistant bacteria globally. The widespread application of broad-spectrum antibacterial agents can lead to changes in antimicrobial resistance[1,5]. Therefore, data on the frequency and resistance characteristics of SBIs in patients hospitalized for COVID-19 are needed[6]. In a small number of studies on COVID-19 and SBIs, only the bacterial distribution was specified, but there are no enough data on resistance rates. Therefore, these studies are not sufficient to guide the empirical use of antibacterial agents[3,4].

Hoping that the information obtained can guide empirical treatment planning and contribute to the prevention of future antimicrobial resistance, in our study, we aimed to evaluate the

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frequency of SBI agents and resistance conditions in patients hospitalized in ICUs with the diagnosis of COVID-19.

METHODS

This retrospective study was carried out in 514 patients who were treated for lung health and diseases and who were anesthetized in ICU in the Sakarya University Education and Research Hospital, Turkey, in March 2019 and February 2021, with a proven diagnosis of COVID-19. The study protocol was approved by the local ethics committee of the Sakarya University School of Medicine.

Demographic data of the patients, onset time of the disease and duration of hospitalization, current antibiotic treatment, and laboratory findings were analyzed retrospectively.

The results of tracheal aspirate culture, blood culture, and urine culture obtained from COVID-19 patients – at least 2 days after their admission to the ICU – were examined microbiologically. Wound and stool cultures were not included. Other sterile body fluid culture samples were not included in the evaluation when they were few in number.

All endotracheal aspirate samples were gram-stained and studied by microbiologists. If the same agent was grown in at least two sets of blood cultures taken simultaneously, it was accepted as a factor. Blood culture samples taken in a single bottle were not included in the study.

All samples taken to the laboratory were planted in sheep blood agar, chocolate agar, and eosin methylene blue agar. Plates were incubated at 35–37°C for 24–48 h. Identification of the isolates grown at the end of the incubation at species level was made by MALDI-TOF/MS. Antimicrobial susceptibility studies of the isolates identified at the species level were performed using VITEK 2. Along with clinical findings, colony growth over 10^4 CFU/mL in tracheal aspirate culture and over 10^5 CFU/mL in urine culture was considered as infection.

Antimicrobial susceptibility results were evaluated according to EUCAST criteria.

All patients with microorganisms in culture were evaluated clinically. Samples considered as contamination/normal flora organisms and interpreted as colonizations were not accepted as SBI.

RESULTS

A total of 514 patients, 324 men and 190 women, hospitalized in ICUs with a mean age of 68.9 years, were included in our study.

All patients who had reverse transcription-polymerase chain reaction testing confirmed the diagnosis of COVID-19-associated pneumonia and were transferred to ICU according to standard criteria. The average number of days of hospitalization in ICU was 8.3. Notably, 336 (65.4%) of patients died during the 1 month follow-up; 173 of them (33.7%) were transferred to the service by improving their general condition. Tracheal aspirate, blood, or urine cultures were obtained from 369 patients (71.8%). Bacterial reproduction was detected in at least one sample in 171 (33.3%) of all patients. Simultaneous reproduction was detected in more than one sample of 26 (5.0%) patients. Secondary fungal infection was developed in six patients (1.1%). There was a growth of pathogenic microorganisms in at least one of the tracheal fluid or blood cultures of 108 (21%) patients. While 106 (61.9%) patients who developed SBI resulted in death, 65 (38%) of them were transferred to another service. The results are shown in Table 1.

Reproduction was detected in the blood culture of 72 patients who developed SBI, in the tracheal aspirate culture of 45 patients, and in the urine culture of 80 patients.

Table 1. Demographic, clinical, and microbiological characteristics of patients.

Sex Men/women, n (%)	324 (63)/190 (37)		
	Mean	**Median**	**SD (min/max)**
Age	68.9	71	13.9 (20/100)
Hospital day	8.35	6	8.9 (1/89)
Survival status, n (%)	Exitus	Transferred to non-ICU	Already stayed in ICU
	336 (65.4)	173 (33.7)	5 (1)
	Present	**Absent**	**Not taken as an example**
Reproduction in any sample	171(33.3)	198 (38.5)	145 (28.2)
Reproduction in tracheal aspirate	45 (8.75)	27 (5.25)	442 (86)
Reproduction in blood culture	72 (14)	199 (38.7)	243 (47.3)
Reproduction in urine culture	80 (15.6)	147 (28.6)	287 (55.8)

The tracheal aspirate culture was obtained from 72 (14%) patients according to the criteria of high fever, C-reactive protein, procalcitonin, white blood cell increase, consolidation areas on lung X-ray, and increased secretion amount. There was no reproduction in seven (9.7%) of them. Upper respiratory tract flora elements were produced in 20 (27.8%) of them. *Acinetobacter baumannii* was the most common microorganism grown in tracheal aspirate culture in 13 (18%) patients with COVID-19. This was followed by *Klebsiella pneumoniae* in nine (12.5%) patients and by *Pseudomonas aeruginosa* in five (6.9%) patients. Multiple microorganisms were detected in five patients.

Blood culture was obtained from 271 (52.8%) of patients. Reproduction was not detected in 199 (73.4%) patients, while reproduction was observed in 72 (26.5%) of them. When the factors grown in blood culture were evaluated, *Staphylococcus epidermidis* was grown in 19 (7%) patients. The second most common was *K. pneumoniae* in 15 (5.5%) patients. Subsequently, *A. baumannii* was detected in six (2.2%) patients and *Enterococcus faecalis* in five (1.8%) patients.

Urine culture was obtained from 227 (44.1%) patients. Reproduction was found in 80 (35.7%) of them. *Escherichia coli* was isolated in 21 (9.3%) and *K. pneumoniae* in 21 (9.3%) of the urine samples with reproduction. *E. faecalis* was detected in 11 (4.8%) of the samples.

The distribution of microorganisms is shown in Table 2.

When all sample regions were included, *K. pneumoniae* (26.3%) was the most frequently isolated agent from all samples, followed by *E. coli* (15.7%), *A. baumannii* (12.9%), and *S. epidermidis* (11.1%). *Candida* reproduction was observed in only six of patients (Table 2).

When the antimicrobial resistance of the bacteria grown was evaluated, all *A. baumannii* strains were found to be resistant to meropenem, imipenem, ciprofloxacin, levofloxacin, piperacillin, tazobactam, ceftazidime, and cefepime, while 68% of the strains were resistant to amikacin, 68% to trimethoprim sulfamethoxazole, and 88% to gentamicin. All strains were found to be susceptible only to colistin.

In *P. aeruginosa*, all strains were found to be susceptible to amikacin, whereas colistin resistance was detected in only one strain. Also, 64.2% (nine strains) of strains were found to be resistant to piperacillin and tazobactam, 35.7% (five strains) to gentamicin, and 28.5% (four strains) to imipenem.

In *K. pneumoniae*, 6.72% of strains were resistant to imipenem, 62.2% to ceftazidime, and 66.7% to ciprofloxacin.

The results of antimicrobial susceptibility of *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* strains are shown in Table 3.

Methicillin resistance was observed in 64% of *Staphylococcus* strains. All strains were susceptible to vancomycin and teicoplanin.

DISCUSSION

It is a long-known fact that viral infections increase the frequency of SBIs and the morbidity and mortality rates. For example, in the 2009 H1N1 influenza pandemic, bacterial infection secondary to viral infection was developed in 20–30% of patients, which increased mechanical ventilation and mortality rates[7]. In the light of these experiences from past viral outbreaks, empirical antibiotic treatment is initiated in more than 70% of hospitalized COVID-19 patients[4]. However, studies showing the frequency of SBIs in COVID-19, the diversity of microorganisms, and their resistance status are very few[2,5,6]. To know the frequency and resistance rates of secondary infections that significantly increase mortality, especially during pandemic periods, antibiotic selection is of great importance in terms of patient management, correct use of resources, and prevention

Table 2. Types of microorganisms that grow according to sample location.

n (%)	Tracheal aspirate	Blood	Urine	Total (n/%)
Acinetobacter baumannii	13	6	5	22/12.9
Klebsiella pneumoniae	9	15	21	45/26.3
Pseudomonas aeruginosa	5	2	2	9/5.3
Staphylococcus aureus	4	4	1	9/5.3
Staphylococcus epidermidis	–	19	1	20/11.7
Enterococcus faecalis	–	5	11	16/9.4
Escherichia coli	1	1	27	29/15.7
Corynobacterium spp	3	5	–	8/4.7
Candida albicans	–	2	4	6/3.5
Staphylococcus hominis	–	4		4/2.4
Pseudomonas aeruginosa+Enterococcus faecalis	2	–	1	3/1.8

Table 3. Antimicrobial susceptibility results of microorganisms.

	Acinetobacter baumannii n: 28 (%)	*Pseudomonas aeruginosa* n: 14 (%)	*Klebsiella pneumoniae* n: 45 (%)	*Escherichia coli* n: 21 (%)
Colistin	28 (100)	13 (92.9)	–	–
Tmp/smx	9 (32.1)	–	24 (53.3)	14 (66.7)
Amikacin	8 (28.6)	14 (100)	21 (46.7)	19 (90.5)
Gentamycin	3 (10.7)	9 (64.3)	27 (60)	17 (81)
Tigecycline	3 (10.7)	–	–	–
Cefepim	–	11 (78.6)	24 (53.3)	12 (57.1)
Imipenem	9 (32.1)	10 (71.4)	24 (53.3)	21 (100)
Ceftazidime	3 (10.7)	9 (64.3)	19 (42.2)	14 (66.7)
Meropenem	9 (32.1)	8 (57.1)	33 (73.3)	33 (73.3)
Ciprofloxacin	8 (28.6)	8 (57.1)	23 (51.1)	8 (38.1)
Levofloxacin	8 (28.6)	7 (50)	22 (48.9)	13 (61.9)
Ertapenem	9 (32.1)	–	31 (68.9)	31 (68.9)

of resistance development[6,8]. In the post-COVID era, increased global antibiotic resistance could be a potential public health problem[9]. Therefore, collecting clinical data on secondary infections and antibiotic resistance is important and almost critical[1,7].

In our study, we investigated the frequency of SBIs and resistance status in patients hospitalized in ICUs with the diagnosis of COVID-19.

The rate of secondary respiratory tract and/or bloodstream infection was found to be 21% in our study. Our results are coherent with reports from available cohorts, where the proportion of patients with secondary infections ranged from 5 to 30%[3,4,10,11]. In our study, the urinary system infection rate was found to be 15.6%, bloodstream infection rate was 14%, and respiratory system infection rate was 8.4%. *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* in tracheal aspirate culture; coagulase-negative staphylococus, *K. pneumoniae*, and *A. baumannii* in blood culture; and *E. coli*, *K. pneumoniae*, and *E. faecalis* in urine culture were the most common microorganisms. When we observed the resistance rates, *A. baumannii* was resistant to most antibiotics except colistin. *P. aeruginosa* strains were resistant to most antibiotics except amikacin, colistin, cefepim, and imipenem. In *K. pneumoniae*, the highest meropenem sensitivity (73%) was observed, and there was a resistance to most of the remaining antibiotics.

The rates of resistant bacteria in ICUs vary between countries and hospitals. The results of our study were similar to the period before COVID-19, except for the high rate of *A. baumannii*. The reason for the high rates of *Acinetobacter* can be explained by the longer hospitalization period and more steroid and immunosuppressive use in COVID-19 patients.

In early studies, SBI was reported in 5–27% of all COVID-19 patients and 13.5–44% of those hospitalized patients in the ICU[3,12,13]. Among the pathogens responsible for the development of these infections, it is observed that multidrug resistant bacteria are common[1,4]. Among the microorganisms isolated from patients, *A. baumannii*, carbapenem-resistant enterobacteriaceae, *K. pneumoniae*, *P. aeruginosa*, *Candida albicans*, and *Candida glabrata* that produce broad spectrum β-lactamase (ESBL) are the most frequently detected microorganisms[13,14]. However, according to earlier studies, changes can be observed in the frequency of detected microorganisms. For example, in the Zhou et al.'s[6] study, SBI was detected in 28 (14%) of 191 patients, and 27 of them died. Coexistence of SARS-CoV-2 and bacterial infection increases mortality at a high rate[1,2,3]. In our center, 62% of patients who developed SBI resulted in death.

Currently available clinical data show that patients infected with SARS-CoV-2 have a lower bacterial or fungal co-infection rate than other viral infections. The reason for this may be that the healthcare personnel providing care and treatment services are highly adapted to the use of personal protective equipment and hand disinfection, because secondary infections may develop due to microorganisms carried by medical equipment or personnel, especially in patients hospitalized in ICUs. Another reason may be the use of empirical antibiotics in the early period of SARS-CoV-2 infection.

Viral infections damage the respiratory tract both histologically and functionally[1]. Damage to ciliary cells can lead to impaired mucociliary clearance, increased adhesion of bacteria to mucins, and increased colonization of bacteria in the

airway[15]. Increased susceptibility to secondary infections may vary depending on the severity of the disease, the type of virus, and the bacterial strain. However, the prevalence and microbiology of concomitant bacterial infections in patients with SARS-CoV-2 infection are not yet fully understood. The number of studies on this subject is relatively small[1,2,5,6].

The strength of our study is that it provides comprehensive data on this subject because we do not have sufficient information, including antibiotic resistance status. Our study has some limitations. The first of these limitations is that invasive procedures such as central venous or arterial catheters cannot be clearly defined and distinguished. Since the majority of patients were critically ill, they had a catheter and were mechanically ventilated. Second, since we are a pandemic center, we could not establish a control group with non-COVID-19 patients from the same period. Therefore, we could not certainly state the percentages of bacterial growth in our patients caused by COVID-19.

CONCLUSIONS

Potential management interventions should be urgently considered to support reduced antimicrobial prescribing during the COVID-19 pandemic. There are no guidelines for which antibiotic to choose in which clinical situations. Knowing the regional and global causative microorganisms and resistance characteristics will increase the success of the pandemic struggle and reduce the common resistance problem in the post-COVID period by contributing to the selection of appropriate antibiotics[9].

As a result, we think that our study can be useful in choosing empirical antibiotics in the COVID-19 pandemic and in reducing the mortality that may occur with secondary infection.

AUTHORS' CONTRIBUTIONS

ÖA, YA: Writing – original draft & editing, Investigation, Software, Formal Analysis, Data curation, Visualization. **EÖŞ, FŞ, MK, AFE:** Investigation. **ÖA**: Methodology.

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ORIGINAL ARTICLE

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Assessment of serum endocan levels in patients with beta-thalassemia minor

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SUMMARY

OBJECTIVE: Beta-thalassemia minor is a blood disease caused by a hereditary decrease in beta-globin synthesis, frequently leading to hypochromic microcytic anemia. Formerly called endothelial cell-specific molecule 1, endocan is a proteoglycan released by vascular endothelial cells in many organs. Our aim was to investigate the relationship between the beta-thalassemia minor patients and the healthy control group in terms of serum endocan level.

METHODS: The study was performed in a total of 80 subjects. They were divided into two groups, the beta-thalassemia minor group (n=40) and the healthy control group (n=40). Serum endocan levels, age, sex, body mass index value, and tobacco use data of these groups were compared.

RESULTS: No statistically significant difference was detected between the two groups in terms of age, sex, and body mass index values (p>0.05). Endocan levels were measured to be 206.85±88.1 pg/mL in the beta-thalassemia minor group and 236.1±162.8 pg/mL in the control group with no significant difference between the groups in terms of serum endocan levels (p>0.05).

CONCLUSIONS: In our study, there was no change in endocan level in beta-thalassemia minor. This might be because serum endocan levels are affected by multi-factorial reasons. Serum endocan levels may be altered secondarily to decreased beta-globin chain, increased sympathetic activity due to anemia, or platelet dysfunction induced by oxidative stress in beta-thalassemia minor. Further multicenter studies involving more patients are necessary to demonstrate this.

KEYWORDS: Thalassemia. Anemia. Endothelial cells. Proteoglycan.

INTRODUCTION

Thalassemias are a group of hereditary genetic disorders characterized by the reduction or absence of synthesis of one or more globin chains in the hemoglobin structure. Beta-thalassemia minor (BTM) is characterized by hypochromic microcytic anemia caused by hereditary decrease in beta-globin synthesis[1]. Studies have reported that cerebrovascular and cardiovascular ischemic events might occur less in BTM patients. We believe that this might be due to decreased serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels, mild anemia (due to low blood viscosity and microcytosis), low incidence of arterial hypertension, and hypofunctional platelets (PLTs) in BTM patients[2-6]. Formerly called endothelial cell-specific molecule 1, endocan is a proteoglycan released by vascular endothelial cells in many organs. Its synthesis is increased by proangiogenetic molecules and pro-inflammatory cytokines. Serum endocan levels are increased in endothelium activation (inflammation) and neovascularization. Studies have shown that serum endocan levels are increased in diseases with endothelial dysfunction such as chronic kidney disease, diabetes mellitus (DM), acute coronary syndrome (ACS), sepsis, and hypertension[7-11].

We aimed to show whether serum endocan levels affect the less frequent occurrence of cardiovascular and cerebrovascular ischemic events in BTM patients. Therefore, we investigated the serum levels of endocan, a marker of endothelial dysfunction between the BTM patients and the healthy control group, which was never performed in the literature earlier.

METHODS

Study group

This was a prospective, cross-sectional, case-controlled study that was initiated after a written approval was obtained from the Bezmialem Vakif University ethics committee and all subjects (approval no:71306642-050.01.04). A total of 80 subjects (40 patients with BTM and 40 in the control group) with similar age, gender, and body mass index (BMI) who applied to Bezmialem Vakif University, Faculty of Medicine, Internal

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Medicine Outpatient Clinic and met the inclusion criteria were included in the study.

Inclusion and exclusion criteria

Subjects aged between 18 and 65 years were included in the study. Patients with hypertension, DM, ischemic cardiac disease, cerebrovascular disease, chronic inflammatory autoimmune disease, malignancy, chronic pulmonary disease, thyroid dysfunction, and severe obesity (BMI >35 kg/m^2), and patients who are pregnant or lactating were excluded from the study.

Group classification

The mean corpuscular volume (MCV) values of the BTM subjects included in the study were <80 fL, and hemoglobin alpha 2 (HbA2) was ≥3.5%. Subjects who were not anemic (Hb≥13 g/dL for men and ≥12 g/dL for women) with normal MCV values were taken into the control group.

Blood assay

Venous blood samples collected into gel biochemistry tubes from all subjects between 8:00 a.m. and 9:00 a.m. after 12 h of fasting were centrifuged for 10 min at 2,100 rpm. Sera of all subjects were separated and aliquoted into Eppendorf tubes and kept at -80°C until the study day. On the study day, the serum samples were brought to room temperature, and routine biochemistry tests and endocan levels were studied in the medical biochemistry laboratory. Complete blood count (CBC) was studied on the same day when the blood was collected from the volunteers into tubes with K2EDTA.

The concentration of serum endocan level was measured by a specific commercial ELISA kit according to the manufacturer's instructions. Concentrations were determined using a spectrophotometric microtiter plate reader (Varioskan Flash Multimode Reader; Thermo, Waltham, MA, USA) at 450 nm optical density. Results were expressed as pg/mL.

Complete blood count was analyzed using Sysmex XT 1800i device (ROCHE2011, Kobe, Japan). Biochemical analyses were performed using COBAS 8000 device (ROCHE-2007, Tokyo, Japan) and COBAS-C system kits. Thyroid hormone levels were measured using Advia Centaur (Advia-2013-Tarrytown, USA) kits. Hemoglobin electrophoresis was performed by high-performance liquid chromatography method using the Shimadzu 20-A (Shimadzu-2013, Kyoto, Japan) device.

Furthermore, the homeostasis model assessment of insulin resistance index (HOMA-IR), a measure of insulin sensitivity, was calculated by multiplying the fasting insulin concentration (μU/mL) and fasting glucose concentration (mmol/L) and divided by 22.5[12].

Statistical analysis

Statistical Package for Social Sciences (SPSS), Windows 20.0 software, was used for the statistical analysis of the data. Quantitative variables were expressed as mean±standard deviation. The Mann–Whitney U test was used for the comparison of the quantitative variables between two groups. Student's t-test was used to compare the parametric variable between the patient and the control group, and the chi-square test was used for the comparison of the categorical variables. Bivariate correlation analyses were performed using Spearman's test. A p-value of <0.05 was considered to be significant.

RESULTS

A total of 80 subjects, 31 men (38.8%) and 49 women (61.2%), were included in the study. Mean age of the subjects was 36.99±12.29 years. There was no statistically significant difference between the mean age and mean BMI between the BTM and control group (p>0.05). Erythrocyte count [red blood cell count (RBC)], red distribution width (RDW), and HbA2 and hemoglobin F (HbF) values of the BTM group were statistically significantly higher than the control group (p=0.001). Hemoglobin (Hg) and hematocrit (Hct), HbA, and MCV values of the BTM group were found to be statistically significantly lower than the control group (p=0.001). No statistically significant difference was detected between the PLT and white blood cell (WBC) values of the groups (p>0.05; Table 1).

Endocan values were measured to be 206.85±88 pg/mL in the BTM group and 236.1±162.8 pg/mL in the control group with no statistically significant difference between the two groups (p>0.05; Table 1). Erythrocyte sedimentation rate (ESR) was statistically significantly lower in the BTM group compared with the control group (p<0.05; Table 1).

No statistically significant difference was detected between the groups in terms of serum glucose, creatinine, TC, TG, HDL-C, LDL-C, glycosylated hemoglobin (HbA1c) and HOMA-IR, transferrin saturation (TS), and thyroid-stimulating hormone (TSH) measurements (p>0.05; Table 1).

When the serum endocan levels, sex, and tobacco use data were compared between the two groups, no statistically significant difference was detected (p>0.05; Table 2).

No statistically and regression analysis-based significant relationship was detected between the BTM (+) and the control group in terms of age-endocan and, HbA-endocan relationships (p>0.05; Table 3).

Table 1. Evaluation of demographic characteristics and blood assay results in the beta-thalassemia minor and control group.

Groups			Test value
BTM (+) (n=40) Mean±SD		Control (n=40) Mean±SD	p
Age (year)	38.83±12.26	35.15±12.20	0.183
BMI (kg/m^2)	26.96±3.59	26.31±4.33	0.466
WBC (10^3/μL)	7.33±1.57	7.56±1.5	0.496
RBC (10^6/μL)	5.67±0.69	4.88±0.47	0.001*
Hb (g/dL)	11.28±1.38	13.91±1.58	0.001*
Hct (%)	36.22±4.12	41.66±4.32	0.001*
MCV (fL)	63.96±3.64	85.35±3.86	0.001*
PLT (10^3/μL)	238.9±61.47	251.28±49.88	0.326
RDW (%)	15.81±2.58	13.51±2.90	0.001*
HbA (%)	93.26±2.03	97.28±0.29	0.001*
HbA2 (%)	5.24±0.64	2.69±0.32	0.001*
HbF (%)	1.47±1.56	0±0.02	0.001*
Endocan (pg/mL)	206.85±88.1	236.1±162.8	0.321
CRP (mg/dL)	0.48±0.54	0.58±0.62	0.675
ESR (mm/h)	6.5±6.33	8.13±6.9	0.038*
Glucose (mg/dL)	94.03±26.42	88.93±9.92	0.421
Creatinine (mg/dL)	0.76±0.12	0.8±0.13	0.100
TC (mg/dL)	172.25±36.67	188.30±46.22	0.116
Triglyceride (mg/dL)	105.63±58.54	116.65±67.95	0.679
HDL-C (mg/dL)	51.58±12.93	54.53±27.98	0.497
LDL-C (mg/dL)	99.3±25.84	111±36.61	0.103
HbA1c (%)	5.41±0.54	5.34±0.31	0.462
AST (U/L)	19.85±10.55	19.13±6.24	0.977
ALT (U/L)	23.25±26.57	23.13±23.73	0.747
Transferrin saturation rate (%)	29.13±14.5	29.23±13.14	0.974
TSH (μIU/mL)	1.51±0.82	1.74±0.74	0.185
FT3 (pmol/L)	4.64±0.55	4.62±0.53	0.882
FT4 (pmol/L)	12.74±1.46	13±1.37	0.423
HOMA-IR	2.65±3.44	2.48±3.22	0.108

*Statistically significant. SD: standard deviation; BTM: beta-thalassemia minor; BMI: body mass index; WBC: white blood cell count; RBC: red blood cell count; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; PLT: platelets; RDW: red blood cell distribution width; HbA: hemoglobin A; HbA2: hemoglobin alpha 2; HbF: hemoglobin F; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HbA1c: glycosylated hemoglobin; AST: aspartate amino transferase; ALT: alanine amino transferase; TSH: thyroid stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine, HOMA-IR: homeostasis model assessment insulin resistance.

Table 2. Evaluation of serum endocan levels by gender and smoking status between beta-thalassemia minor and control group.

			Endocan test value	
			Mean±SD	p
BTM (+)	Sex	Men (n=15)	188.8±80	0.369
		Women (n=25)	217.6±92.5	
Control	Sex	Men (n=16)	203.5±74	0.599
		Women (n=24)	257.8±200	
BTM (+)	Smoking	None (n=20)	205.0±93.9	0.899
		Present (n=20)	208.6±84	
Control	Smoking	None (n=20)	230.7±149	0.860
		Present (n=20)	241.5±178.8	

SD: standard deviation; BTM: beta-thalassemia minor.

Table 3. Relationship of endocan with age and laboratory results between the beta-thalassemia minor (+) and control group.'

	BTM (+)		Control	
	Endocan		Endocan	
	r	p	r	p
Age	0.182	0.260	0.141	0.385
HBA	-0.083	0.612	0.295	0.650
Glucose	0.077	0.639	0.101	0.535
Creatinine	-0.104	0.524	0.045	0.784
TC	0.101	0.534	0.67	0.681
Triglyceride	-0.061	0.710	0.109	0.504
LDL-cholesterol	0.034	0.835	0.087	0.592
HDL	0.185	0.253	-0.018	0.91
ESR	0.093	0.570	0.008	0.960
HbA1C	0.153	0.345	-0.209	0.196
HOMA-IR	-0.068	0.677	-0.095	0.558

SD: standard deviation; BTM: beta thalassemia minor; HbA: hemoglobin A; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; ESR: erythrocyte sedimentation rate; HbA1c: glycosylated hemoglobin; HOMA-IR: homeostasis model assessment insulin resistance.

DISCUSSION

In this study, we aimed to detect the serum endocan levels and the relationship of this marker with anthropometric, metabolic, and biochemical parameters between BTM patients and healthy control group. In our literature search, we did not find a study evaluating the relationship of endocan levels with BTM patients and anemia.

Studies have reported that cerebrovascular and cardiovascular ischemic events might occur less in BTM patients. It was

shown that this might be because of decreased TC, LDL-C, and TG levels, mild anemia (due to low blood viscosity and microcytosis), low incidence of arterial hypertension, and hypofunctional PLTs in BTM patients[2-6]. However, the exact pathophysiology is yet to be illuminated. Endocan is believed to be released from endothelium and plays an important role in vascular diseases, inflammation, and endothelium-dependent pathological disorders[7-11]. In this study, we aimed to show that endothelium damage might be less in patients with BTM, and, as a result, whether the serum endocan levels are low or not.

Some studies have detected low serum, TC, and LDL-C levels in BTM, and it was caused by excessive LDL-C uptake by the reticuloendothelial system due to accelerated erythropoiesis in BTM[4,13]. In their study, Agarwal et al. found that the incidence of myocardial infarction is lower in BTM, and this was thought to be caused by low blood viscosity due to mild anemia and microcytosis in BTM[3] . In their study, Cikrikcioglu et al. detected low levels of CD-40 ligand and soluble P-selectin and, PLT activation factors in BTM. They stated that hypofunctional PLT in BTM might have protective effects against cardiovascular and cerebrovascular ischemic diseases[6]. In a study, patients with the history of thromboembolic cerebrovascular event, cerebrovascular disease percentage, and arterial blood pressure were found to be less. Thus, the reason for cerebrovascular ischemic diseases occurring less in BTM was due to the effect of low arterial blood pressure[5].

In general, it is thought that cardiovascular and cerebrovascular ischemic events in patients with BTM might be low due to anemia, microcytosis, hyperviscosity, low TC and LDL-C levels, and incidence of low arterial pressure. In some other studies, serum endocan levels were higher in patients diagnosed with ACS and essential hypertension. It was stated that endocan might be a novel marker showing endothelial dysfunction, and the increase in the endocan level might be a risk for cardiovascular and ischemic events[10,14].

In their study on lipid profiles in patients with BTM and control subjects, Hashemieh et al. detected significantly lower levels of TC and LDL-C in BTM. In the same study, when the subjects were classified by age, no significant difference was detected between the BTM patients and the control group for the subjects aged <25 years in terms of cholesterol levels [i.e., TC, TG, LDL-C, HDL-C, and very low-density lipoprotein cholesterol (VLDL-C)]. In the BTM group aged from 26 to 40 years, TC and LDL-C levels were significantly lower, and in the BTM group aged >40 years, only LDL-C level was significantly lower[4]. In their study, Selek et al. detected significantly lower LDL-C levels in BTM patients, and did not detect a significant difference for TC, TG, and HDL-C levels[15]. Thus, it can be shown that regional ethnicity, diet types, age, and genetic differences may affect the cholesterol levels in BTM. In our study, there was no statistically significant difference between the groups in terms of TC, TG, LDL-C, and HDL-C levels, because our study is a single-centered study with less number of patients.

Normally, inflammatory cytokines [i.e., interleukin 1 (IL-1) and, tumor necrosis factor alpha (TNF-α)] secreted after sepsis and inflammation induce endocan expression. The blood levels of this proteoglycan might reflect treatment response, and the presence and the severity of inflammation. Therefore, endocan has a role in endothelial dysfunction during the inflammatory process[9,16]. We also believe that endocan levels might be low in BTM as the inflammatory process is not involved in the etiopathogenesis of BTM.

Anemia might increase the activity of sympathetic nervous system[17,18]. Selek et al. detected that oxidative stress is increased due to anemia in BTM[15]. Oxidative stress increase in BTM might impair the structure of membrane glycoproteins in PLT and might cause a decrease in the PLT functions. PLT dysfunction might be congenital or acquired in BTM. Oxidative stress may also cause acquired PLT dysfunction[6].

Botta et al. found the endocan level to be high in transfusiondependent beta-thalassemia patients (beta-thalassemia major)[19]. This may be due to direct or indirect endothelial damage caused by chronic hemolysis and chronic iron overload in patients with β-thalassemia major. In our study, however, there was no significant change in endocan level in BTM patients. According to the study of Botta et al., it can be considered that our study was designed in a different thalassemia group, and there was no chronic hemolysis and iron overload. This indicates that endocan release might be affected in BTM due to congenital globin chain impairment, sympathetic activity, PLT dysfunctions, oxidative stress, or other factors.

Chronic inflammation also plays a role in the pathogenesis of atherosclerosis. In clinical practice, ESR is an acute phase marker used to show inflammation. In their cohort study in 1,679 patients, Natali et al. have shown that high ESR level is an independent marker of coronary atherosclerosis associated with cardiac mortality[20]. ESR might increase in macrocytosis, and chronic disease anemias developed secondarily to many illnesses (e.g., infection, inflammation, and malignancy) usually due to underlying diseases. ESR is decreased in polycythemia and some erythrocyte disorders (i.e., sickle cell anemia, microcytosis spherocytosis, and acanthosis)[21,22]. In our study, ESR was found to be significantly lower in the BTM group compared with the control group ($p<0.05$). We believe that, this is caused by the increase of total erythrocyte count in BTM.

The most important limitations of our study were as follows: (1) less number of subjects in the BTM and the control group were involved and this is a single-centered study and (2)

we could have used more objective parameters to demonstrate endothelial dysfunction (flow-mediated vasodilation).

CONCLUSION

In our study, no significant difference was detected between the BTM patients and the control subjects in terms of serum endocan levels. This might be because serum endocan levels are affected from multi-factorial reasons. Serum endocan levels may be altered secondarily to decreased beta-globin chain, increased sympathetic activity due to anemia, or PLT dysfunction induced by oxidative stress in BTM. Further multicenter studies involving more patients are necessary to demonstrate this.

AUTHORS' CONTRIBUITIONS

NK: Data curation, Writing – review & editing. **MZ:** Conceptualization, Investigation, Project administration, Writing – original draft. **OFO:** Formal Analysis, Methodology. **CK:** Resources, Validation. **MK:** Supervision, Data curation, Software. **MÇ:** Validation, Visualization, Funding acquisition.

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ORIGINAL ARTICLE

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Relationship of the prenatal psychosocial profile with postpartum maternal duties and newborn care†

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SUMMARY

OBJECTIVE: This study aimed to determine the relationship of the prenatal psychosocial profile with postpartum maternity duties and newborn care.
METHODS: This descriptive and correlational study was conducted on 154 pregnant women.
RESULTS: It was determined that pregnant women had low-stress levels, high social support from their husbands and other people, and moderate self-esteem. Participants relying on power in coping with maternity duties in the postpartum period and satisfaction with maternity and newborn care were found to be high.
CONCLUSION: Adaptation to the postpartum period facilitates the transition to maternity and increases satisfaction with newborn care.
KEYWORDS: Psychological stress. Social seupport. Self concept. Newborn. Postpartum period. Prenatal care.

INTRODUCTION

Prenatal psychosocial health is related to health behaviors, maternal health, and birth outcomes[1]. The woman's feeling of a new living being in her body and the reaction of the family and social environment to pregnancy form the basis of psychosocial reactions because psychosocial behaviors have the potential to directly or indirectly affect the outcome of pregnancy[2]. Women who perceive themselves as positive during pregnancy have been determined to have healthier pregnancies and better birth outcomes[1]. Stress experienced during pregnancy, inadequate social support, and low self-esteem are known to have adverse effects on pregnancy outcomes[2].

It is crucial for parents to adapt to the postpartum period to get used to both the transition process to maternity and the participation of a new individual in the family[3]. This process may be a transition period during which it is easy for many families to adapt and which increases communication within the family, or it may also be a period during which they may have difficulty in the care of their infants, besides their own needs, and feel inadequate[4]. It is determined that receiving education on pregnancy, postpartum, and newborn care process eases women's adaptation to pregnancy and maternity[5] and increases their self-confidence levels to cope with maternal duties[6].

In the interviews conducted by health care personnel to meet the psychosocial needs of the mother in the postpartum period, they should talk and evaluate the woman's adaptation to the new role, her birth experience, and changes that occur with the participation of a newborn in the family. In fact, relatives who will contribute to family members in the postpartum period should be informed about how they should provide support and about the progress of the process[7,8].

METHODS

Aim and type of the study

This descriptive and correlational study was planned to determine the relationship of the prenatal psychosocial profile (PPP) with postpartum maternal duties and newborn care.

Research questions

- What are the stress, social support (spouse and other people), and self-esteem levels of pregnant women?
- Does the PPP affect the level of coping with postpartum maternal duties?

Does PPP affect satisfaction in postpartum motherhood and newborn care?

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Population and sample of the study

The study was conducted in a family health center in Istanbul between June 2017 and March 2018. The sampling formula with the known population was used to determine the sample size of the study, and the number of pregnant women to be included was calculated. A total of 154 pregnant women, who did not have any chronic- or pregnancy-related diseases, who were able to understand, comprehend, and answer the questions, and who accepted to participate in the study, were included in the study. This article complies with the Strengthening the Reporting of Observational Studies in Epidemiology guideline.

Data collection tools used in the study

The researcher developed a descriptive information form as a result of the literature review, and it consists of 25 questions, which include sociodemographic and obstetric characteristics. PPP, which was developed by Curry et al.[9] in the United States, is a Likert-type assessment tool consisting of four subscales (i.e., stress, social support from partner, social support from other people, and self-esteem) and a total of 44 items, which evaluate women's perceived stress during pregnancy, the social support they receive from their partners and environment, and their self-esteem. The validity and reliability studies of the scale in Turkish version were conducted by Günaydın and Zengin (2019). Cronbach's α value in the stress subscale was found to be 0.78 in the study by Curry et al.[9], and it was found to be 0.75 in the Turkish version. In their study, Curry et al.[9] found that Cronbach's α values for the social support from partner and social support from other people subscales were found to be 0.93 and 0.95, respectively. In the Turkish version of the assessment tool, Cronbach's α values for the social support from partner and social support from other people subscales were found to be 0.94 and 0.96, respectively. Cronbach's α value for self-esteem subscale was found to be 0.89 in the study by Curry et al.[9] and 0.80 in the Turkish version.

The Postpartum Period Descriptive Questionnaire form developed by the researcher as a result of the literature review consists of 13 questions.

The Postpartum Self-Evaluation Questionnaire (PSEQ), which was developed by Lederman and Weingarten in 1981 to evaluate the adaptation of women in the postpartum period to maternity, is a four-point Likert-type scale with seven subscales and a total of 82 items. The validity and reliability studies of the scale in our country were carried out by Tasci and Mete[10]. In the study, the subscales relying on power in coping with maternal duties and satisfaction with maternity and newborn care were used.

The way followed in the collection of the study data

The stages of collecting the study data are presented in Figure 1.

Data analysis

Statistical Package for Social Science (SPSS), version 22, was used for statistical analysis while evaluating the findings obtained in the study. Descriptive statistical methods (i.e., mean and standard deviation) and *t*-test were used for data evaluation.

Ethics

To use the PSEQ, written permission was obtained from the person adapting the scale, Istanbul Public Health Directorate (approval date: February 20, 2017; approval number: 64222187-060.99), and Istanbul University Cerrahpaşa Clinical Research Ethics Committee (approval date: December 06, 2019; approval number: A-01). In accordance with the Declaration of Helsinki, written and verbal information about the study and nature of the study was provided to the participants, and their written consent was obtained.

RESULTS

The mean age of the pregnant women participating in the study was 28.73±5.22 (min: 18, max: 41) years, and the mean age of their spouses was 32.52±5.26 (min: 22, max: 50) years. It was found that the majority of both the women (58.4%) and their spouses (63%) had an education level of over 8 years, and more than half (60.4%) of the pregnant women did not work.

According to the scores received by the participants from the PPP subscales, it was determined that their stress levels were low (16.16±4.24), social support received from their partners was high (55.32±12.07), social support received from their environment was high (53.52±12.40), and their self-esteem was moderate (23.93±2.78) (Table 1).

The participants were determined to score 19.61±5.30 from the PSEQ subscale of "relying on power in coping with maternal duties" and 15.82±3.31 from the subscale of "satisfaction with maternity and newborn care." The results showed that postpartum adaptation was high (Table 1).

When the mean scores of the PPP subscales were compared according to the women's age, no significant difference was determined between the groups in terms of the mean scores of all four dimensions (Table 2).

The mean scores of the PPP subscales were compared according to the participants' education duration and employment status, while a difference was not determined between the

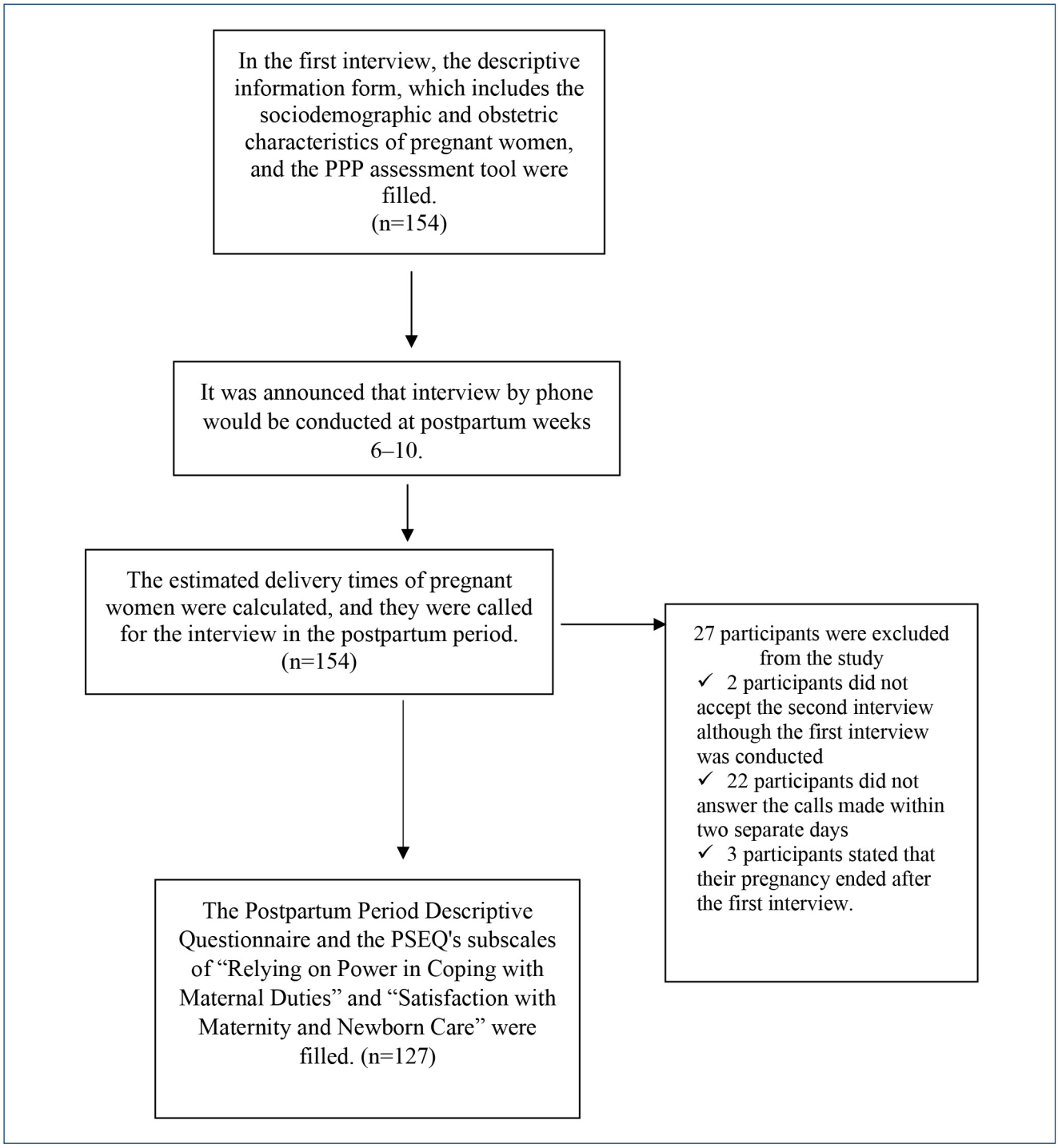

Figure 1. Stages of the collection of the study data.

groups in terms of the mean stress, social support from partner, and self-esteem scores. However, a significant difference was determined between the mean scores of the social support from other people subscale (Table 2). The mean score of the social support from other people subscale of the women who worked and had the education of more than 8 years was found to be significantly lower than that of the women who did not work and had the education of 8 years and below (Table 2).

When the mean scores of the PPP subscales were compared according to the income level of the women, it was found that

Table 1. Mean scores that the participants received from the prenatal psychosocial profile and two subscales of the postpartum self-evaluation questionnaire.

	Mean	SD	Min.	Max.
PPP subscales (n=154)				
Stress	16.16	4.24	11	35
Social support from partner	55.32	12.07	12	60
Social support from other people	53.52	12.40	11	60
Self-esteem	23.93	2.78	16	33
PSEQ subscales (n=127)				
Relying on power in coping with maternal duties	19.61	5.30	14	45
Satisfaction with maternity and newborn care	15.82	3.31	13	32

the difference between the groups' mean scores in the self-esteem subscale was not significant and that it was significant in the stress, social support from partner, and social support from other people subscales (Table 2). The mean scores of the social support from partner and social support from other people subscales of the women with insufficient income were significantly lower than those of the women with a high-income level, and their mean scores in the stress subscale were significantly higher (Table 2).

When the mean scores of the PPP subscales were compared according to the women's status of receiving postpartum support, no significant difference was determined according to the groups' mean scores of the stress and self-esteem subscales (Table 2). The mean scores of the social support form partner

Table 2. Comparison of the prenatal psychosocial profile and subscale mean scores according to the characteristics of the participants.

	PPP and its subscales			
Characteristics	**Stress Mean±SD**	**Social support from partner Mean±SD**	**Social support from other people Mean±SD**	**Self-esteem Mean±SD**
Age groups				
29 years and below (n=70)	14.71±3.85	57.08±11.22	53.87±11.08	24.04±2.92
Above 29 years (n=84)	15.38±4.15	53.85±12.61	53.23±13.46	23.84±2.68
Test value (t)	*−1.025*	*1.662*	*0.320*	*0.437*
p-value	*0.307*	*0.095*	*0.749*	*0.663*
Education time (years)				
8 years and below (n=64)	14.84±3.27	55.60±11.80	56.25±11.51	23.54±3.21
Above 8 years (n=90)	15.24±4.48	55.12±12.32	51.58±12.73	24.21±2.41
Test value (t)	*0.641*	*0.246*	*2.331*	*−1.395*
p-value	*0.523*	*0.806*	***0.021***	*0.166*
Employment status				
Employed (n=61)	15.54±3.91	55.16±11.86	50.49±14.07	24.44±2.61
Unemployed (n=93)	14.77±4.07	55.63±12.27	55.51±10.79	23.60±2.85
Test value (t)	*1.159*	*−0.133*	*−2.368*	*1.844*
p-value	*0.248*	*0.894*	***0.020***	0.067
Income level				
Insufficient income (n=38)	16.60±4.09	50.36±15.38	49.18±13.77	23.57±3.02
Sufficient income (n=116)	14.57±3.88	56.94±10.34	57.94±11.63	24.05±2.70
Test value (t)	*2.757*	*−2.461*	*−2.529*	*−0.907*
p-value	***0.007***	***0.017***	***0.012***	*0.366*
Status of receiving support in the postpartum period				
Yes (n=142)	16.09±4.26	56.46±11.21	54.33±11.96	23.95±2.71
No (n=12)	17.08±4.12	41.83±15.11	43.91±13.65	23.75±3.69
Test value (t)	*−1.137*	***3.274***	***2.859***	*0.239*
p-value	*0.257*	***0.007***	***0.005***	*0.812*

Bold values indicate significance.

and social support from other people subscales of the women who could receive support in the postpartum period were determined to be significantly higher than those of the women who could not receive support in the postpartum period (Table 2).

When the mean scores of two subscales of the PSEQ were compared according to the women's age, education time, employment, and economic status, no significant difference was determined between subscales' mean scores (Table 3).

DISCUSSION

It is known that some sociodemographic variables, such as age, low income, and low education level, pose a risk to health and form a basis for psychological and physical problems[11]. It is known that psychosocial health improves as the education levels of pregnant women increase[12]. In the present study, while no relationship was found between the education level and stress, social support from partner, and self-esteem, pregnant women with a low education level were found to feel more social support from their environment. In the literature, there are studies which support the results of the present study and which do not detect any relationship between the education level and stress[13]. Furthermore, in contrast to the results of the present study, there are also studies indicating that the social support perception[11,14] and self-esteem scores of people with a high educational level are high[14]. This difference is due to the population on whom the study was conducted and the measurement tools are different.

Table 3. Comparison of the mean scores of two subscales of the postpartum self-evaluation questionnaire according to the descriptive characteristics of the participants.

	PSEQ and its subscales	
Characteristics	**Relying on power in coping with maternal duties Mean±SD**	**Satisfaction with maternity and newborn care Mean±SD**
Age groups		
29 years and below (n=55)	19.85±5.89	15.40±2.83
Above 29 years (n=72)	19.43±4.84	16.15±3.62
Test value (t)	*0.445*	*−1.271*
p-value	*0.657*	*0.206*
Education time (years)		
8 years and below (n=47)	19.27±4.66	15.44±2.42
Above 8 years (n=80)	19.22±5.79	16.05±3.73
Test value (t)	*−0.548*	*−0.990*
p-value	*0.584*	*0.324*
Employment status		
Employed (n=53)	19.39±5.27	15.98±3.87
Unemployed (n=74)	19.77±5.35	15.71±2.86
Test value (t)	*-0.391*	*0.443*
p-value	*0.697*	*0.659*
Income level		
Insufficient income (n=32)	19.96±5.55	15.90±3.36
Sufficient income (n=95)	19.49±5.24	15.80±3.31
Test value (t)	*0.936*	*0.156*
p-value	*0.664*	*0.876*

While working life provides women with a higher quality of life and a higher level of welfare, it may cause more problems with pregnancy[12]. In the literature, there are studies determining that there is no relationship between stress[13] and social support and employment status[11]. In the current study, working women were found to receive less social support from their environment than nonworking women. This situation suggests that working pregnant women should be supported.

This study determined that women with low income were more stressful than those with sufficient income. In contrast to the present study, Yehia et al.[14] and Curry et al.[9] determined that women with a low income had lower stress levels than others. In the study by Akın[13], no relationship was found between income level and stress experienced during pregnancy. Different study results are observed in the literature. However, the current study data could be interpreted as low-income women experience more stress due to unfavorable living conditions caused by economic conditions. Furthermore, as a result of the present study, it was revealed that women with low income received higher social support from their partners and the environment. Unlike the result of the present study, Mermer et al.[11] did not found a relationship between income level and social support. Different sample characteristics may have led to differences in the results obtained.

Social support meets the person's needs, such as showing interest to someone and feeling affection, respect, and intimacy to someone, and also positively affects his/her physical and mental health[12]. The present study determined that the mean scores of the social support from partner and social support from other people subscales of the women, who could receive support in the postpartum period, were higher. In the studies in the literature, the stress level and depressive symptoms were found to decrease as social support increased, and the mother–infant attachment was also found to be better in

the postpartum period[15,16]. The social support that women receive from their partners and environment during pregnancy is observed to affect both the pregnancy and the postpartum period positively. Empowering women about support during pregnancy and the postpartum period should not be ignored[17].

Women with good adaptation to the maternal role during pregnancy are more likely to rely on power in coping with postpartum maternal duties[3,17]. Factors, such as education level, occupation, income level, and social security, which determine the sociological status of women, are very significant factors affecting the maternal role of women[18]. In this study, it was found that the score obtained from the subscale relying on power in coping with maternal duties did not change according to the age, education time, employment, and economic status of the women. In the studies, it was determined that mothers, who had high income[5,18] and education levels, who were at an advanced age, and who worked[5,18], had better adaptation to the maternal role. In order to facilitate adaptation to the process of transition to maternity, it is vital that health care providers know this process. Thus, during the follow-up period, both the transition process of pregnant women to maternity will be facilitated[8].

Mothers need counselling services for their own care and the care of their newborns in the postpartum period[6]. In this study, women's satisfaction with maternity and newborn care was observed not to change with their age, education time, employment, and economic status. However, detecting and preventing the problems in the early postpartum period are essential in terms of contributing to the health of both the mother and the newborn[4].

In the postpartum period, the woman is in the process of adaptation to the maternal role. During this period starting from pregnancy, the woman learns maternal behaviors and accepts maternal identity. It is stated that women who exhibit a positive attitude have higher satisfaction with the maternal role. Moreover, education and counselling service provided during pregnancy and the postpartum period are important in terms of facilitating the woman's transition to the maternal role and ensuring that she is able to cope with difficulties related to newborn care. The studies demonstrated that satisfaction of such women with maternity and newborn care was higher[3].

CONCLUSION

When an evaluation was made in terms of the PPP subscales, it was found that the stress levels of pregnant women were low, social support received from their partners and other people was high, and their self-esteem was moderate. When an evaluation was made in terms of the postpartum PSEQ subscales, it was found that the participants relying on power in coping with maternal duties and satisfaction with maternity and newborn care were high.

Limitations

Since the results of this study represent a particular sample group, they cannot be generalized to all pregnant women. The fact that the data were collected in a certain period of time is one of the limitations of the study. Since the expressions in the scales are based on individual statements, the margin of error should be considered.

AUTHORS' CONTRIBUTIONS

SG: conceptualization, methodology, data collection, writing—original draft, and writing—review and editing. **NZ:** conceptualization, methodology, writing—original draft, and writing—review and editing.

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ORIGINAL ARTICLE

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Low-dose paclitaxel modulates the cross talk between the JNK and Smad signaling in primary biliary fibroblasts

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SUMMARY

OBJECTIVE: The objective of this study was to explore the molecular mechanism underlying the occurrence of benign bile duct stricture and the target of low-dose paclitaxel in the prevention of benign bile duct stricture.

METHODS: Under the stimulation of transforming growth factor beta 1, the expression of collagen type I and connective tissue growth factor were detected on isolated primary fibroblasts. The phosphorylation levels of JNK and Smad2L were detected using Western blot. The effect of low-dose paclitaxel on the transforming growth factor beta 1-induced inhibition of type I collagen and connective tissue growth factor expression and JNK and Smad2L phosphorylation was also observed.

RESULTS: Transforming growth factor beta 1 induced the secretion of type I collagen and connective tissue growth factor as well as JNK phosphorylation in biliary fibroblasts. The JNK inhibitor or siRNA-Smad2 inhibited the transforming growth factor beta 1-induced secretion of type I collagen and connective tissue growth factor. Low-dose paclitaxel inhibited the expression of type I collagen induced by transforming growth factor beta 1 and may inhibit the secretion of collagen in biliary fibroblasts.

CONCLUSION: The activation of JNK/Smad2L induced by transforming growth factor beta 1 is involved in the occurrence of benign bile duct stricture that is mediated by the overexpression of type I collagen and connective tissue growth factor, and low-dose paclitaxel may inhibit the phosphorylation of JNK/Smad2L.

KEYWORDS: Bile duct. Fibroblasts. JNK. Smad. Paclitaxel.

INTRODUCTION

Anastomotic stricture of the bile duct is one of the main complications after liver transplantation, and the incidence rate is as high as 24.9%[1]. The incidence of biliary stricture after living-donor liver transplantation in children is as high as 10–35%[2]. Bile duct stricture severely affects the life and treatment of patients. Therefore, in order to effectively prevent benign bile duct stricture, it is necessary to explore the molecular mechanism underlying the occurrence of benign bile duct stricture.

Recent studies have found that low-dose paclitaxel can prevent tissue fibrosis[3-5], and clinically, paclitaxel coating has been used to prevent the occurrence of luminal narrowing of blood vessels[6]. Preliminary animal studies of this subject showed that low-dose paclitaxel-coated stents can inhibit the activation of biliary-enteric anastomosis fibroblasts and the secretion of collagen through slow topical administration, but the specific molecular mechanism is still unclear[7]. In this study, we aimed to explore the molecular mechanism underlying the occurrence of benign bile duct stricture. At the same time, the effect of low-dose paclitaxel on the inhibition of type I collagen and connective tissue growth factor (CTGF) secretion investigated in relation to the molecular mechanisms was also obtained.

METHODS

Isolation, culture, and identification of primary biliary fibroblasts

The isolation, culture, and identification of primary biliary fibroblasts were performed according to the earlier reported procedure from Wistar rats[8]. The following experiments were performed using the fifth generation cells, and the cells were starved with 10 mL/L fetal calf serum for 12 h before any interventions. SP600125 (Calbiochem, USA) inhibits JNK phosphorylation. Paclitaxel was purchased from Sigma, and transforming growth factor beta 1 (TGF-β1) was purchased from Peprotech. The experiment was approved by the Animal Ethical Board for Biomedical Experiments of The First Affiliated Hospital of Xi'an Jiaotong University.

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siRNA transient transfection of siRNA into cells

Transfection was carried out using Lipofectamine 2000 in accordance with Invitrogen's manufacturer protocol. Western blotting was performed for 48 h after transfection to determine the inhibitory effect on protein expression.

Enzyme-linked immunosorbent assay test

Following centrifugation at 1,000 r/min for 5 min at 4°C, the supernatant of medium was collected and stored in a refrigerator at –20°C. The type I collagen enzyme-linked immunosorbent assay detection kit (MyBioSource, San Diego, CA, USA) was used to detect the corresponding protein expression level in accordance with the manufacturer's instructions.

Western blotting for the detection of protein levels

The proteins were separated using SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane. The membrane was blocked with 0.1% BSA in PBS for 1 h at room temperature and then incubated with the primary antibodies in PBST overnight at 4°C. The membrane was incubated with a goat anti-rabbit secondary antibody (1:2,000) for 1 h at room temperature. Scion NIH image analysis software was used to detect the intensity of the protein bands on Western blot in order to determine the expression level of the target protein.

Statistical analysis

The experimental results are expressed as mean±standard deviation. One-way analysis of variance was used to compare multiple groups of data, while the comparison between the two groups was performed by LSD test using SPSS version 17.0 software. $p<0.05$ was regarded as statistically significant, and $p<0.01$ was regarded as the difference of high statistical significance.

RESULTS

Transforming growth factor beta 1 induces collagen secretion in biliary fibroblasts

According to Table 1 and Figure 1A, TGF-β1 induced the secretion of type I collagen in biliary fibroblasts in a dose-dependent manner, and 10 ng/mL TGF-β1 increased the level of type I collagen secreted by 2.07 times compared with the control group ($p<0.01$).

Transforming growth factor beta 1 induces the expression of connective tissue growth factor in biliary fibroblasts in a dose-dependent manner

As shown in Figure 1B, TGF-β1 induced the CTGF expression in a dose-dependent manner, and we found that 10 ng/mL TGF-β1 increased the CTGF expression by 3.28 times as compared to the control group ($p<0.01$).

Transforming growth factor beta 1 induces phosphorylation of JNK in biliary fibroblasts in a dose-dependent manner

As shown in Figure 1C, 3 ng/mL TGF-β1 increased the phosphorylation level of JNK as compared to the control group ($p<0.05$), whereas 5 ng/mL TGF-β1 increased the phosphorylation level of JNK by 3.85 times compared with the control group ($p<0.01$).

Transforming growth factor beta 1-induced phosphorylation of JNK mediates the activation of Smad2L

In comparison with the control group, the phosphorylation of Smad2L was induced in the TGF-β1 group ($p<0.05$). In contrast, compared with the TGF-β1 group, the JNK inhibitor SP600125 inhibited the phosphorylation of Smad2L mediated by TGF-β1 ($p<0.05$; Figure 1D).

Selection of Smad2 gene-specific siRNA for biliary fibroblasts via Smad2 siRNA interference screening

As shown in Figure 1E, the Western blot following 48 h post-transfection indicates that *Smad2* siRNA-3 had a significant inhibitory effect on the negative control group and the blank control group ($p<0.01$), while *Smad2* siRNA-1 and -2 had no obvious inhibitory effect. Therefore, we chose *Smad2* siRNA 3 for follow-up experiments.

Table 1. Transforming growth factor beta 1 induces collagen secretion in biliary fibroblasts.

Concentration of Transforming growth factor beta 1 (ng/mL)	0	1	3	10
Concentration of collagen (ng/mL)	2.37±0.76	2.51±0.50	3.35±1.05	4.90±1.06*

*The difference is statistically significant ($p<0.01$) in comparison with the control group.

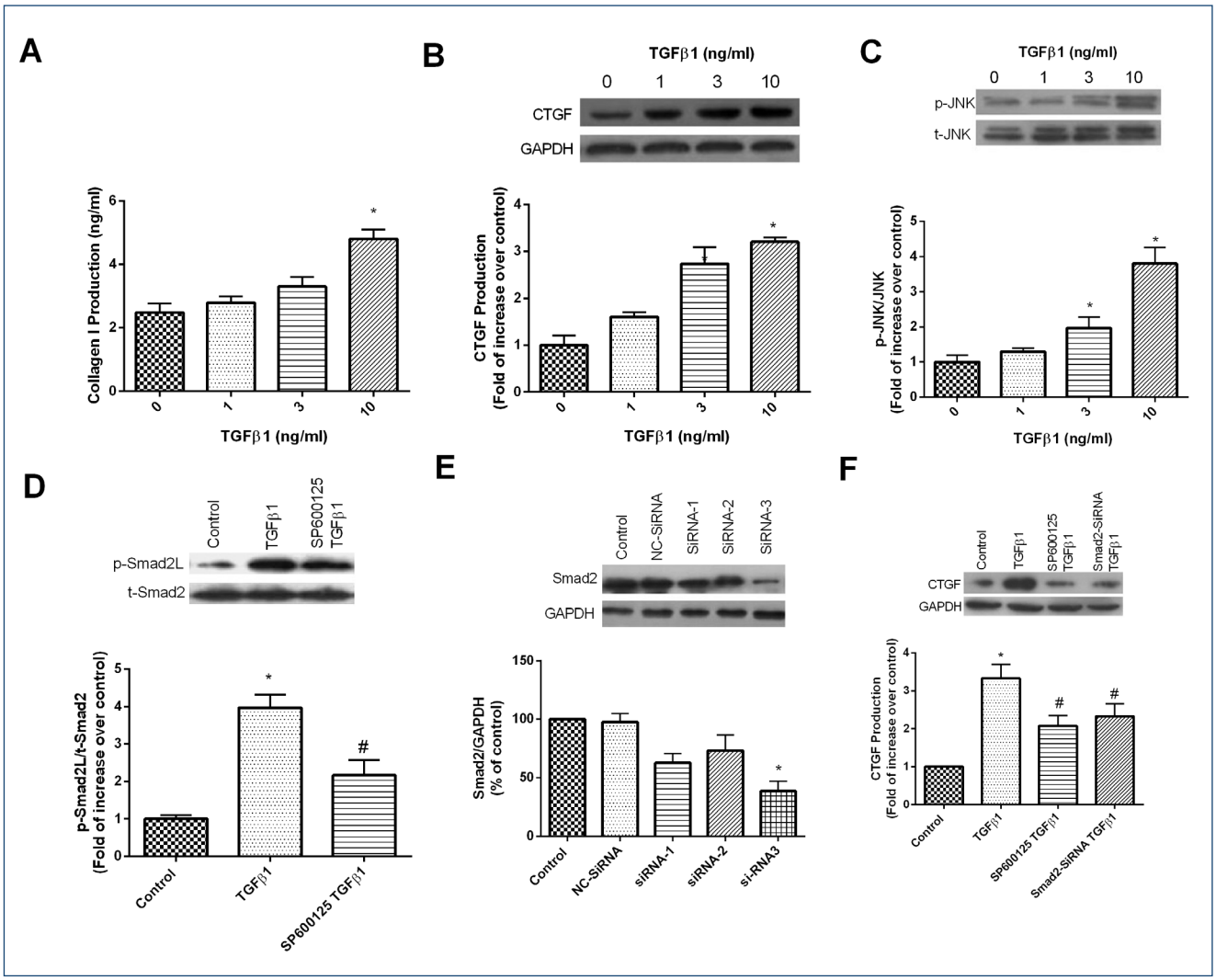

Figure 1. Transforming growth factor comparison: (A) collagen production in biliary fibroblasts, (B) connective tissue growth factor expression in biliary fibroblasts, (C) phosphorylation of JNK in biliary fibroblasts, (D) phosphorylation of JNK mediates the phosphorylation of Smad2L, (E) inhibitory effect of three siRNAs on Smad2 protein, and (F) JNK/Smad2L signaling pathway mediates the secretion of connective tissue growth factor. *The difference is statistically significant ($p<0.01$) in comparison with the control group. #The difference is statistically significant ($p<0.05$) in comparison with the transforming growth factor beta 1 group.

Transforming growth factor beta 1 activates the JNK/Smad2L signaling pathway and mediates the secretion of connective tissue growth factor

Compared with the control group, the expression level of CTGF in the TGF-β1 group was enhanced ($p<0.01$). The JNK inhibitor SP600125 inhibited the expression level of CTGF that was mediated by TGF-β1 as compared with the TGF-β1 group. In the *Smad2* siRNA group, TGF-β1-mediated CTGF expression was also inhibited. Compared with the JNK inhibitor group and the Smad2 siRNA group, no significant difference in the expression level of CTGF mediated by TGF-β1 was observed. Thus, it might be suggested that TGF-β1 mediates the expression of CTGF by activating the JNK/Smad2L signaling pathway (Figure 1F).

Transforming growth factor beta 1 induces the phosphorylation of JNK and Smad2L and mediates the expression of type I collagen

Figure 2A shows that compared with the control group, the expression level of type I collagen of the TGF-β1 group was increased ($p<0.01$). The JNK inhibitor SP600125 inhibited the expression level of type I collagen mediated by TGF-β1. In the *Smad2* siRNA group, the expression level of type I collagen mediated by TGF-β1 was also inhibited. Therefore, these

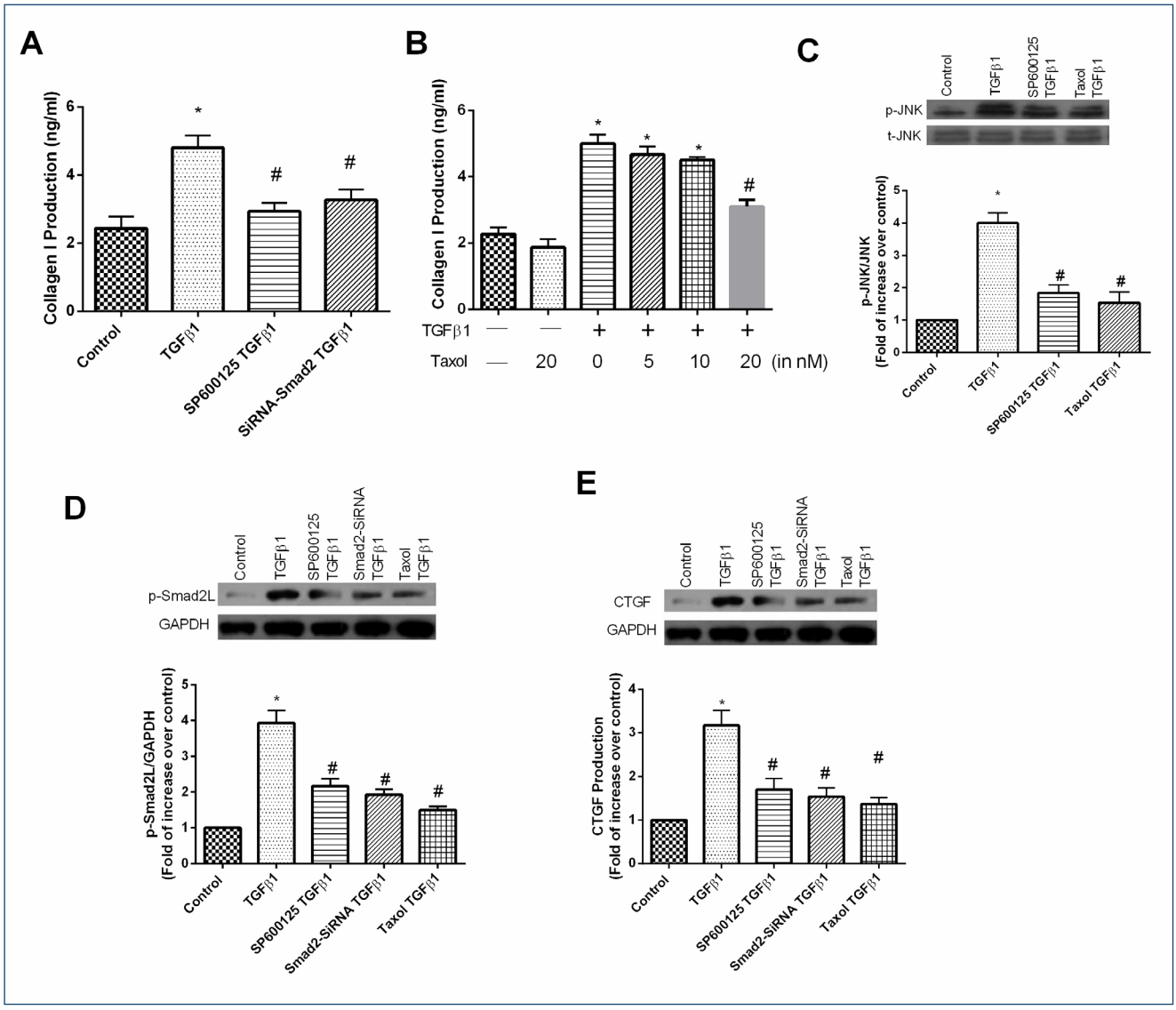

Figure 2. Molecular mechanism explained that (A) transforming growth factor beta 1 induces the phosphorylation of JNK and Smad2L and mediates the secretion of type I collagen, (B) paclitaxel inhibits the expression of type I collagen induced by transforming growth factor beta 1, (C) JNK phosphorylation induced by transforming growth factor beta 1, (D) transforming growth factor beta-mediated phosphorylation of Smad2L, and (E) transforming growth factor beta 1-induced connective tissue growth factor expression. *The difference is statistically significant ($p<0.01$) in comparison with the control group; #The difference is statistically significant ($p<0.05$) in comparison with the transforming growth factor beta 1 group.

findings suggest that TGF-β1 mediates the expression of type I collagen by activating the JNK/Smad2L signaling pathway.

Paclitaxel inhibits transforming growth factor beta 1-mediated secretion of type I collagen in biliary fibroblasts in a dose-dependent manner

As shown in Figure 2B, 10 ng/mL TGF-β1 significantly increased the secretion of type I collagen in biliary fibroblasts as compared with the control group. Paclitaxel dose-dependently inhibited the TGF-β1-mediated secretion of type I collagen in biliary fibroblasts. Particularly, 20 nmol/L paclitaxel significantly reduced the secretion of type I collagen in biliary fibroblasts compared with the TGF-β1 group, suggesting that 20 nmol/L paclitaxel has a significant inhibitory effect on TGF-β1-induced type I collagen secretion in biliary fibroblasts. In the absence of TGF-β1 stimulation, paclitaxel has no significant effect on type I collagen of biliary fibroblasts (compared with the blank control group, $p>0.05$). Therefore, 20 nmol/L paclitaxel was chosen for follow-up experimental research.

Effect of paclitaxel on JNK phosphorylation induced by transforming growth factor beta 1

The results in Figure 2C indicate that paclitaxel can significantly inhibit the level of JNK phosphorylation induced by TGF-β1 ($p<0.05$, compared with the TGF-β1 group). However, its effect was not statistically different compared with the JNK inhibitor group ($p>0.05$).

Effect of paclitaxel on transforming growth factor beta 1-mediated phosphorylation of Smad2L

In order to explore whether paclitaxel can inhibit the phosphorylation of Smad2L induced by TGF-β1, the results in Figure 2D indicate that paclitaxel can significantly inhibit the phosphorylation of Smad2L induced by TGF-β1 ($p<0.05$, compared with the TGF-β1 group). Nevertheless, there is no statistical difference in its effect when compared with the JNK inhibitor group or siRNA group ($p>0.05$).

Paclitaxel inhibits the transforming growth factor beta 1-mediated expression of connective tissue growth factor in biliary fibroblasts

To examine whether paclitaxel can inhibit TGF-β1-induced expression of CTGF, the results in Figure 2E indicate that paclitaxel can significantly inhibit the TGF-β1-induced secretion of CTGF compared with the TGF-β1 group, and its effect is similar to that in the TGF-β1 group. Nevertheless, there was no statistical difference when compared with the JNK inhibitor group or the siRNA group ($p>0.05$), indicating that paclitaxel inhibits CTGF secretion possibly via the suppression of the phosphorylation of JNK and Smad2L.

DISCUSSION

A growing body of evidence suggests that non-Smad pathways are involved in the transduction of TGF-β signals. These non-Smad pathways can be directly mediated by TGF-β or can involve in the regulation of TGF-β/Smad signal transduction, thereby bestowing TGF-β with multiple biological functions[9]. Recently, Lessard et al. found that JNK can adjust skeletal muscle remodeling through Smad2[10]. Shapira et al. reported that increased phosphorylation of JNK can increase the translation and activation of Smad2/3 induced by TGF-β[11]. Smads protein is composed of conserved MH1, MH2, and intermediate linking regions and a central mediator of TGF-β signal transduction. TGF-β type I receptor (TβRI) phosphorylates the COOH-terminal serine residues of Smads protein activated by membrane receptors, and the intermediate linking region can be phosphorylated by Ras-related kinases, such as ERK, JNK, P38, and CDK4[12,13]. TGF-β1 not only directly mediates the phosphorylation of the COOH-terminal serine residues of Smads by activating the TβRI but also promotes the activation of Ras-related kinases through TGF-β activated kinase 1 and mediates the phosphorylation of Smads protein linking region to coordinate TGF-β1 signal transduction[13]. The TGF-β1-mediated Ras-related kinase-dependent Smad2/3 intermediate linking region promotes not only cell proliferation but also tissue fibrosis. Abe et al. found that ERK1/2 contributed to the increase of the collagen synthesis mediated by TGF-β1 in cardiac fibroblasts, which was accompanied by phosphorylation of the intermediate linking region of Smad2, while the loss of ERK significantly down-regulated the expression of collagen[14]. In this study, TGF-β1 induced the expression of CTGF and type I collagen in a dose-dependent manner, accompanied by increased phosphorylation of JNK and Smad2L, and inhibition of phosphorylation of JNK or Smad2L could reduce the expression of CTGF and type I collagen. JNK inhibitors can also inhibit the phosphorylation of Smad2L induced by TGF-β1, suggesting that the TGF-β1-induced expression of CTGF and type I collagen in biliary fibroblasts is mediated by the JNK/Smad2L pathway. Hence, TGF-β1/JNK/Smad2L is involved in the occurrence of benign bile duct stricture.

Recent studies suggest that low-dose paclitaxel (10–50 nM) is able to prevent tissue fibrosis by regulating the TGF-β1/Smads signaling pathway and inhibiting the phosphorylation of Smad2/3 to reduce the deposition of collagen[15-17]. The results of this study suggest that low-dose paclitaxel inhibits the TGF-β1-induced secretion of collagen in a dose-dependent manner, accompanied by the inhibition on phosphorylation of JNK and Smad2L. Therefore, we found that low-dose paclitaxel-induced inhibitory effect on the secretion of collagen may be achieved by inhibiting the phosphorylation of JNK and Smad2L, and low-dose paclitaxel may regulate the TGF-β1/Smads signaling pathway through JNK to downregulate collagen secretion in human biliary fibroblasts.

CONCLUSION

Our present study demonstrated that activation of JNK/Smad2L induced by TGF-β1 is involved in the occurrence of benign bile duct stricture that is mediated by the overexpression of type I collagen and CTGF, and low-dose paclitaxel may inhibit the phosphorylation of JNK/Smad2L.

AUTHORS' CONTRIBUTIONS

JL, LY: Performed the study, Analyzed the data, Wrote the initial draft of this manuscript. **JS:** Conceptualized and supervised the study, Approved the final version of this manuscript.

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ORIGINAL ARTICLE

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Effect of meteorological factors on first episode and recurrence of primary spontaneous pneumothorax

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SUMMARY

OBJECTIVE: In this study, we evaluated the clinical characteristics and seasonal distribution of patients with primary spontaneous pneumothorax and examined the relationships between meteorological factors and pneumothorax development overall and in terms of first episode and recurrence.

METHODS: The hospital records of 168 pneumothorax patients treated in our clinic between January 2016 and December 2020 were reviewed retrospectively. A cluster was defined as two or more patients with pneumothorax presenting within three consecutive days. Meteorological factors were compared between days with and without pneumothorax patients. This comparison was based on meteorological data from the day of symptom onset (D), the day before symptom onset (D1), and the difference between those days (D–D1). Meteorological data from the index day (D) were also compared between patients with first episode and recurrence of pneumothorax.

RESULTS: The study included 149 (88.7%) men and 19 (11.3%) women. The mean age was 25.02±6.97 (range, 17–35; median, 26) years. Of note, 73 (43.4%) patients underwent surgery. The highest number of patients presented in November (n=19, 11.3%). In terms of season, most presentations occurred in autumn. Humidity was significantly lower on recurrence days compared with first episode (p=0.041).

CONCLUSION: Our results indicated that meteorological factors (i.e., atmospheric pressure, humidity, wind speed, temperature, and precipitation) were not associated with pneumothorax development. By comparing the patients with first episode and recurrence, the humidity was significantly lower in the recurrence group.

KEYWORDS: Pneumothorax. Meteorology. Humidity. Recurrence.

INTRODUCTION

Pneumothorax is characterized by air accumulation in the pleural space followed by lung collapse and is classified into spontaneous, traumatic, or iatrogenic[1]. Primary spontaneous pneumothorax (PSP) usually occurs in young, healthy individuals (aged 10–40 years) in the absence of any underlying lung disease[2]. Recurrence refers to repeated episodes of pneumothorax that occur after an initial episode. Studies have reported the recurrence rates of 13–49% in the first year and above 50% in 5-year follow-up[3]. Rupture of a bleb, bulla, or subpleural pores in the lung apex has been implicated in the pathogenesis of PSP, but the etiological factors remain unclear. Asthenic body type, smoking, young age, and male sex are recognized as risk factors for PSP[4]. Apart from these, genetic causes, environmental factors, and meteorological conditions have also been implicated.

There are publications in the literature demonstrating a relationship between PSP and meteorological factors and air pollution. These studies have investigated factors, such as air pollution, atmospheric pressure (AP), humidity changes, air temperature, daily rainfall, and wind speed, and some have shown a relationship between these factors and the development of spontaneous pneumothorax.

The present study investigated the clinical characteristics and seasonal distribution of PSP patients and evaluated the associations between meteorological factors and PSP development overall and in patients with first episode and recurrence.

METHODS

The hospital records of 168 PSP patients treated in the thoracic surgery department of Recep Tayyip Erdogan University Education and Research Hospital over the 5-year period between January 2016 and December 2020 were reviewed retrospectively. All patients with PSP, who aged between 17 and 35 years, presented to our clinic, and had complete records, were included in the study. Patients, who aged <17 years or >35 years, whose records were incomplete, or who presented to our hospital from outside the region, were excluded.

Demographic data collected from the patients' records included age, gender, PSP side, a first episode or recurrence, length of hospital stay, smoking status, treatment received,

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body mass index (BMI), and reasons for undergoing surgery. The day of PSP occurrence was defined as the day of symptom onset (chest pain and shortness of breath) as determined from the patients' records instead of the day of presentation. Two or more patients presenting with PSP within three consecutive days were defined as a cluster[5].

Wind speed, humidity, temperature, AP, and daily precipitation data were obtained from the National General Directorate of Meteorology. There were no missing data among the datasets. Meteorological factors were compared between days with and without PSP cases based on data from the day of symptom onset (D), the day before symptom onset (D1), and the difference between these days (D–D1). Meteorological factors on the index day (D) were also compared between patients with first episode and those with recurrence of PSP.

Statistical analysis

Analyses were performed using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). Continuous data were presented as mean and standard deviation, and categorical data were presented as numbers and percentages. The distribution of continuous data was evaluated using the Kolmogorov–Smirnov test. The Mann–Whitney U-test was used to analyze relationships between meteorological data and days with and without PSP cases and between days with first episode and recurrence of PSP. A statistical significance level of $p<0.05$ was used for all tests.

RESULTS

A total of 168 patients with PSP treated between January 2016 and December 2020 were included in the study. Of these, 149 (88.7%) were men, and 19 (11.3%) were women. The mean age was 25.02±6.97 (range, 17–35) years, and the median age was 26 years. Of note, 73 (43.4%) patients underwent surgery. The mean length of hospital stay for patients not treated surgically was 6.26±2.33 days, while that of patients who underwent surgery was 10.38±4.14 (median, 9.8) days. The mean BMI of patients was 21.67±3.12, and 118 (70.2%) patients had smoking history. The most common symptoms were chest pain (90.4%), shortness of breath (17.7%), and cough (2.9%). Pneumothorax was on the right side in 103 (61.3%) patients and on the left side in 65 (38.7%) patients. Surgery was performed in 38 (52.1%) patients due to recurrent pneumothorax, in 32 (43.8%) patients due to prolonged air leak, and in 3 (4.1%) patients for other reasons (hemothorax in 2 patients and expansion defect in 1 patient) (Table 1). The highest number of patients presented in November (n=19, 11.3%). In terms of season, most presentations occurred in autumn.

The 168 PSP episodes occurred on 158 different days. A total of 68 (43%) patients presented in 30 PSP clusters. The mean number of cases in each cluster was found to be 2.1±0.6 (range, 2–4 cases).

The AP (hPa), wind speed (km/h), humidity (%), temperature (°C), and amount of precipitation (mm) were evaluated from the meteorological data of 158 days with PSP symptom onset and the 1,668 days without PSP cases. There were no statistically significant differences between days with and without PSP in terms of meteorological parameters on the index day (D), the day before (D1), or the difference between these days (D–D1) (Table 2).

The comparison of the first episode and recurrence of PSP patients in terms of meteorological data showed that humidity was significantly lower on days with recurrence (p=0.041) (Table 3).

DISCUSSION

Apart from the commonly known factors in the etiology of PSP, one of the current topics is the effect of meteorological factors. Studies have suggested possible associations between

Table 1. Characteristics of patients with primary spontaneous pneumothorax (n=168).

	Mean±SD or n (%)
Age	25.02±6.97
Gender	
Male	149 (88.7)
Female	19 (11.3)
Smoker	118 (70.2)
Mean BMI (kg/m^2)	21.67±3.12
Side of PSP	
Right	103 (61.3)
Left	65 (38.7)
Length of hospital stay	
Surgical patients	10.38±4.14
Nonsurgical patients	6.26±2.33
Reason for surgery	
Recurrence	38 (52.1)
Prolonged air leaked	32 (43.8)
Other reasons	3 (4.1)
Number of PSP episodes	
First episode	106 (63.1)
Recurrence (2 or more)	62 (36.9)

BMI: body mass index; SD: standard deviation; PSP: primary spontaneous pneumothorax.

Table 2. Comparison of meteorological factors on days with and without cases of primary spontaneous pneumothorax.

Parameter	Days without PSP (1,668 days) mean±SD	Days with PSP (158 days) mean±SD	p
Wind speed (km/h)			
D	5.23±2.52	5.43±2.68	0.329
D1	5.25±2.53	5.22±2.60	0.878
D–D1	−0.02±3.02	0.22±3.18	0.348
Humidity (%)			
D	58.86±15.51	59.15±17.06	0.821
D1	59.06±15.57	57.09±16.47	0.132
D–D1	−0.21±16.48	2.06±17.66	0.100
Atmospheric pressure (hPa)			
D	1015.36±6.11	1015.55±5.72	0.714
D1	1015.34±6.09	1015.75±5.96	0.426
D–D1	0.02±4.12	−0.20±4.14	0.533
Temperature (°C)			
D	15.89±6.91	15.56±6.88	0.558
D1	15.90±6.91	15.49±6.87	0.469
D–D1	0.00±1.74	0.07±2.01	0.633
Precipitation (mm)			
D	5.70±12.98	6.46±15.55	0.493
D1	5.66±12.90	6.91±16.18	0.258
D–D1	0.01±16.22	−0.45±20.31	0.736

D: index day; D1: 1 day earlier; hPa: hectopascal; SD: standard deviation; PSP: primary spontaneous pneumothorax.

Table 3. Comparison of meteorological factors on days with cases of first episode and recurrence of primary spontaneous pneumothorax.

Parameter	Attack number		p
	First episode (n=106) Mean±SD	Recurrence(n=62) Mean±SD	
Wind speed (km/h)	5.30±2.26	5.57±3.19	0.527
Humidity (%)	61.79±16.36	56.24±17.73	0.041
AP (hPa)	1015.22±5.15	1016.06±6.47	0.355
Temperature (°C)	16.05±6.86	14.57±6.98	0.180
Precipitation (mm)	7.70±18.76	5.86±15.26	0.513

AP: atmospheric pressure; hPa: hectopascal; SD: standard deviation.

meteorological factors and myocardial infarction, asthma, abdominal aortic rupture, facial paralysis, and sudden hearing loss[6]. There are also reports of pneumothorax due to pressure changes while diving and during flights[7].

Humidity is a parameter evaluated in many studies, but most could not demonstrate a relationship with PSP[8,9]. Ozenne et al.[10] reported that spontaneous pneumothorax peaked in winter and summer, and its incidence was significantly associated with low humidity. They stated that dry air results in inadequate humidification of the airways and that the subsequent bronchoconstriction may play a role in the physiopathology of pneumothorax. Bozkurt et al.[11] found that the mean change between daily

maximum and minimum humidity levels was 43.1±13.4% on the days when 95 patients presented with spontaneous pneumothorax and 38.6±16.2% on the other days. In our study, there was no relationship between PSP and humidity, but when the first episode and recurrence patient groups were compared, the humidity level was significantly lower on the days when patients presented with recurrence. Therefore, we believed that a decrease in humidity may be a factor in the development of recurrence.

A relationship between wind speed and spontaneous pneumothorax has been mentioned in some publications. Ogata et al.[8] observed a relationship between PSP and high wind speed in their study involving 110 cases. Schiemann et al.[12] conducted a study on 220 patients and found that high wind speed and low AP were associated with spontaneous pneumothorax. In contrast, Bertolucci et al.[13] reported a relationship between spontaneous pneumothorax and low wind speed. In a series of 195 cases analyzed by Yamac et al.[14], the wind speed was significantly lower on the days when patients presented with spontaneous pneumothorax. In their two-center study, including 494 patients, Daş et al.[15] demonstrated that spontaneous pneumothorax was associated with northern winds and low wind speed and reported that low temperature and sudden temperature changes could also be factors.

The amount of precipitation has also been investigated, though less frequently. In a series of 669 patients, Özpolat et al.[16] found significantly more precipitation on the days when patients presented with spontaneous pneumothorax and in the 2-day period before these days. Similarly, Alifano et al.[5] reported an increase in spontaneous pneumothorax on stormy days, which they defined as thunder and lightning with heavy rainfall.

Similar to other meteorological factors, our literature review yielded different results related to temperature[5,17,18]. Özpolat et al.[16] showed that PSP was associated with low temperature but noted that this might be a cofactor rather than having a direct effect. In another study of 200 patients, Yaksi et al.[19] also found that a reduction in temperature was significantly associated with the development of PSP. Sahinoglu et al.[20] reported that temperature higher than 15°C may contribute to the development of PSP and that when compared with blood group, people with blood group O were more resistant to temperature changes. In a study by Zhang et al.[21], involving 337 patients, an increase in mean temperature and low AP were reported to play a role in PSP development.

In contrast, changes in AP are the most popular area of study, with the most related articles and the largest patient series. In a recent study, Akyıl et al.[22] evaluated 1,097 PSP patients treated in a 4-year period and reported that low pressure and sudden decrease in pressure during the day might be the factors in the development of pneumothorax. In their study, there was a decrease in pressure on 65% of the days on which PSP cases presented and an increase in pressure on 65% of the days with no PSP cases. Another large study by Haga et al.[23] included 1,051 patients with PSP and showed that the AP fell by 0.6 hPa on the days of PSP cases compared with the day before. In our literature review, there were also a substantial number of studies in which no relationship was detected between AP and pneumothorax, as in our study[24].

In their study in 2007, Alifano et al.[5] defined a cluster as a period of 3 days in which two or more patients presented and reported a cluster rate of 84% in their study involving 294 patients. This rate was 70.5% in a 699 case series reported by Özpolat et al.[16]. In these three studies, clustering was associated with low AP. Haga et al.[23] reported 597 clusters in a study of 1,051 cases, with 88% of the patients in these clusters. The mean number of pneumothorax cases per cluster was 3.2 in the study by Alifano and 2.5±0.8 in the study by Özpolat et al. In our study, 43% of the PSP cases formed clusters, and the mean number of cases per cluster was 2.1±0.6.

In terms of seasonality, we noted in our literature review that PSP was most common in autumn and in the months of June and November[11,18]. Similarly, in our study, we found that the number of patients was higher in autumn and November.

The main limitations of the present study are its retrospective design and our inability to conduct multivariate analysis due to the small number of patients.

CONCLUSION

It is clear from many studies conducted on this subject that the relationship between the meteorological factors and the development of pneumothorax has yet to be proven definitively. We believe that detailed studies comparing different predisposing factors with meteorological data will provide more definitive information in the future.

ETHICAL APPROVAL

Approval for this retrospective, descriptive, single-center study was obtained from the Clinical Research Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine Education and Research Hospital (decision no: 2020/193, dated September 3, 2020).

AUTHORS' CONTRIBUTIONS

KT: conceptualization, data curation, formal analysis, investigation, writing – original draft. **GS:** conceptualization, supervision, writing – review & editing.

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ORIGINAL ARTICLE

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Diagnostic and prognostic significance of long noncoding RNA LINC00173 in patients with melanoma

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SUMMARY

OBJECTIVE: A growing volume of literature has suggested long noncoding RNAs (lncRNAs) as important players in tumor progression. In this study, we aimed to investigate the expression and prognostic value of lncRNA LINC00173 (LINC00173) in melanoma.

METHODS: LINC00173 expression was measured in 163 paired cancerous and noncancerous specimen samples by real-time polymerase chain reaction. The correlations between LINC00173 expression with clinicopathological characteristics and prognosis were analyzed by chi-square test, log-rank test, and multivariate survival analysis. Receiver-operating characteristic curves were used for the assessment of the diagnostic value of LINC00173 for melanoma patients.

RESULTS: The expression level of LINC00173 in melanoma specimens was distinctly higher than that in adjacent non-neoplasm specimens ($p<0.01$). Besides, LINC00173 was expressed more frequently in patients with advanced melanoma than in patients with early melanoma. Multivariate assays confirmed that LINC00173 expression level was an independent prognostic predictor of melanoma patients ($p<0.05$).

CONCLUSION: Our data indicated that LINC00173 expression could serve as an unfavorable prognostic biomarker for melanoma patients.

KEYWORDS: Long Noncoding RNA LINC00173. Melanoma. Biomarker.

INTRODUCTION

Human melanoma is one of the most aggressive and frequently diagnosed cancers in humans[1]. In China, melanoma has displayed an increasing trend[2]. Despite the continued advancements in surgical operation program, radiation, and chemotherapy, the therapeutic effectiveness of advanced melanoma has not shown distinct improvements, and the long-term survivals remain dismal[3,4]. The poor prognosis for melanoma patients was due to the high frequency of metastasis and recurrence[5]. Therefore, it is imperative to reveal the mechanisms underlying melanoma progression and identify new biomarkers and therapeutic targets for melanoma patients.

Long noncoding RNAs (lncRNAs) are another class of noncoding RNA with more than 200 nucleotides in length that lack a complete open reading frame (ORF)[6]. Growing studies have demonstrated that lncRNAs are conserved in many animals and specifically expressed in several cellular types and the development of biological progress[7]. LncRNAs play essential roles in several biological processes, and several different functions of lncRNAs in the gene modulation have been identified, such as protein sponges and miRNAs sponges[8,9]. Importantly, growing studies have focused on the potential effects of lncRNAs in tumor progression via the novel mechanism "miRNAs sponge"[10,11]. Moreover, the frequently dysregulated expressing pattern of lncRNAs in clinical tumor specimens is positively correlated with malignancy grades and histological differentiation, which suggests that some functional lncRNAs may be used as potential biomarkers in tumor diagnosis of subclassification and prognostication[12,13]. In recent years, several melanoma-related lncRNAs have been functionally identified and could be used as novel prognostic and diagnostic biomarkers for melanoma patients, such as lncRNA HOTAIR, lncRNA PVT1, and lncRNA CRNDE[14-16].

In this study, we identified a novel melanoma-related lncRNA, i.e., lncRNA LINC00173 (LINC00173), which was first functionally identified in lung cancer by Yang et al[17]. The distinct upregulation of LINC00173 has been confirmed in several tumors, such as gastric carcinoma, esophagus cancer, and breast cancer[18-20]. However, its expression and biological function in tumors remained largely unclear. For the first time, we provided clinical evidence that LINC00173 was highly expressed in melanoma and may be used as a novel diagnostic and prognostic biomarker for melanoma patients.

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METHODS

Patients and tissue samples

The 163 melanoma tissues and their pair-matched nontumor specimens in this study (collected from May 2012 to July 2015) were obtained from patients who underwent radical resections at the first people's hospital of Jinan city. All cases gave informed consent for their samples to be used for our experiments. None of the patients had received radiotherapy or chemotherapy before surgery. All collected tissue samples were immediately snap-frozen and stored for further real-time polymerase chain reaction (RT-PCR). A comprehensive set of clinical parameters was recorded by the follow-up department of our hospital and further used for clinicopathological and prognostic assays. The Ethics Committee of our hospital approved our study.

RNA extraction and qRT-PCR assays

With the use of TRIzol reagent (Invitrogen, Hangzhou, Zhejiang, China), the extraction of total RNA from melanoma specimens and matched nontumor tissues was carried out based on the standard procedures provided by the company. A PrimeScript RT Reagent Kit with cDNA eraser (Takara Biotech, Pudong, Shanghai, China) was used for cDNA synthesis with one microgram of total RNA as a template. RT-PCR was carried out using cDNA primers specific for LINC00173 and mRNA. GAPDH was used as an internal control for LINC00173. Primers for LINC00173 were purchased from Genecopoeia (Xuhui, Shanghai, China). LINC00173: Forward GCCAGCTCTCGGTACCTGGA, LINC00173: Reverse GGATCGCAACATTCCTGCCAAG; GAPDH: ForwardCAAGGTCATCCATGACAACTTTG, GAPDH: ReverseGTCCACCACCCTGTTGCTGTAG.

The relative expression of LINC00173 was expressed as ΔCt=Ct gene – Ct reference, and the $2^{-\Delta\Delta Ct}$ methods were applied for the calculation of the fold change.

Statistical analysis

All statistical analyses were conducted using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). The difference between two independent groups was compared by an independent sample t-test. The chi-square test was used to analyze the relationship between categorical variables. Receiver-operating characteristic (ROC) curve assays were applied to determine the sensitivity and specificity of LINC00173 for melanoma diagnosis. The Kaplan–Meier method was performed for patients' overall survival (OS) and disease-free survival (DFS) to identify independent prognostic factors of significance, and a univariate and multivariate Cox regression was performed. The results were considered to be statistically significant at $p<0.05$.

RESULTS

The differential expression of LINC00173 in melanoma specimens

To determine the expression of LINC00173 in melanoma, qRT-PCR was performed by using fresh melanoma tissues. As presented in Figure 1A, we observed that the expression levels of LINC00173 were distinctly increased in human melanoma specimens compared with adjacent nontumor tissues ($p<0.01$). Besides, LINC00173 was shown to be expressed more frequently in advanced melanoma patients than that in early melanoma patients ($p<0.01$). Our findings indicated LINC00173 as a regulator in melanoma progression.

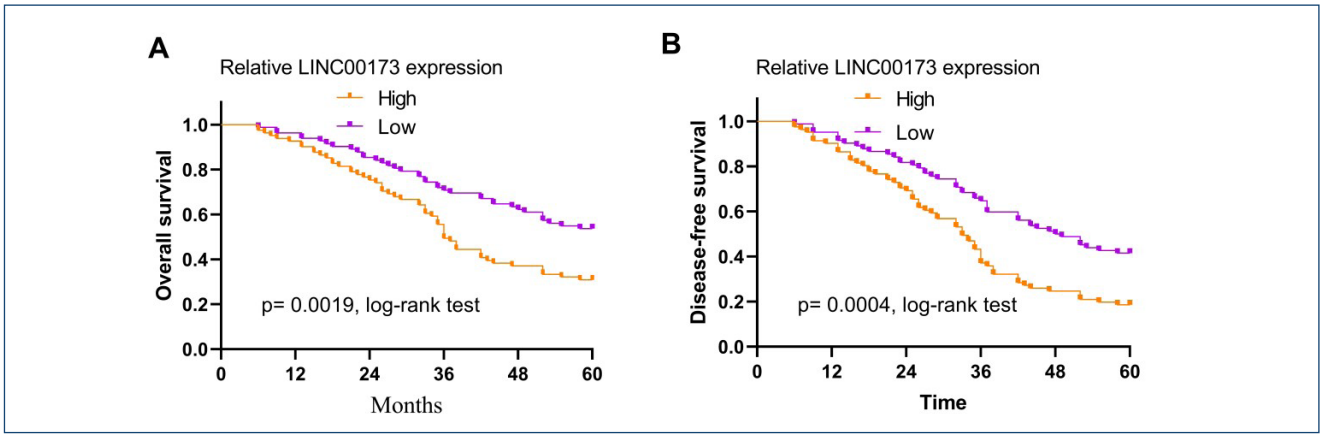

Figure 1. Kaplan–Meier curves of the overall survival (A) and disease-free survival (B) of 163 melanoma patients based on the expression of LINC00173.

Diagnostic value of LINC00173 overexpression in melanoma

To examine the characteristics of LINC00173 as a potential diagnostic biomarker for melanoma patients, ROC curves were constructed on data from all subjects. The results revealed that the area under the curve (AUC) of ROC curve was 0.7695 (95%CI: 0.7172–0.8217, p<0.001, sensitivity: 0.76, specificity: 0.85). Besides, we also performed subgroup analysis, finding that there was a strong separation between the patients with advanced stages and patients with early stages, with an AUC of 0.7451 (95%CI: 0.6512–0.8389; p<0.001) for LINC00173. Overall, our findings indicated LINC00173 as a potential diagnostic biomarker for melanoma patients.

Expression levels of LINC00173 and clinicopathological parameters in melanoma

To investigate the possible significance of LINC00173 expression in clinical progress, all 163 patients were divided into low LINC00173 expression group and high LINC00173 expression group based on the median LINC00173 level in all melanoma specimens. The data from a chi-square test revealed that high LINC00173 expression was positively associated with advanced tumor stage (p=0.026) and lymph node metastasis (p=0.007) (Table 1). However, there was no difference in age and sex (p>0.05).

High expression of LINC00173 correlates with poor prognoses

During the follow-up, 94 patients died, and the median follow-up time was 37±12.3 months. Then, we performed Kaplan–Meier analysis to explore the prognostic value of LINC00173 in melanoma patients. Interestingly, patients with higher LINC00173 expression have shown significantly lower OS (p=0.0019, Figure 1A) and DFS (p=0.0004, Figure 1B) than those with lower LINC00173 expression. To further examine the probability of LINC00173 expression as an independent prognostic parameter of patient outcomes, univariate and multivariate assays were performed. We observed that high LINC00173 expression (OS: p=0.014, DFS: p=0.017, Table 2) was an independent prognostic parameter, indicating poor clinical outcome for melanoma patients.

Table 1. Correlation between LINC00173 expression and clinicopathological characteristics of melanoma patients.

Clinicopathological features	No. of cases	LINC00173 expression		p-value
		High	Low	
Age (years)				
<55	79	35	44	0.182
≥55	84	46	38	
Sex				
Male	92	50	42	0.176
Female	71	31	40	
Tumor thickness (mm)				
≤2	106	47	59	0.062
>2	57	34	23	
Ulceration				
–	110	51	59	0.121
+	53	30	23	
Tumor stage				
I/II	110	48	62	0.026
III/IV	53	33	20	
Lymph node metastasis				
Negative	120	52	68	0.007
Positive	43	29	14	

Table 2. Prognostic factors for overall survival or disease-free survival by univariate and multivariate analysis.

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	p-value	HR (95%CI)	p-value
Overall survival				
Age	0.985 (0.472–1.885)	0.217	–	–
Sex	1.217 (0.665–2.128)	0.185	–	–
Tumor thickness	1.467 (0.885–2.109)	0.118	–	–
Ulceration	1.378 (0.839–1.885)	0.121		
Tumor stage	2.923 (1.327–4.772)	0.013	2.652 (1.175–4.429)	0.018
Lymph node metastasis	3.451 (1.482–5.428)	0.001	3.176 (1.285–4.995)	0.009
LINC00173 expression	2.986 (1.328–4.774)	0.009	2.768 (1.157–4.456)	0.014
Disease-free survival				
Age	1.275 (0.687–1.984)	0.218	–	–
Sex	0.857 (0.478–2.187)	0.182	–	–
Tumor thickness	1.447 (0.852–2.217)	0.118	–	–
Ulceration	1.522 (0.832–2.143)	0.105		
Tumor stage	1.445 (1.442–4.342)	0.009	1.352 (1.322–4.195)	0.015
Lymph node metastasis	3.321 (1.372–5.127)	0.002	3.018 (1.138–4.726)	0.009
LINC00173 expression	2.938 (1.278–4.662)	0.006	2.629 (1.158–4.385)	0.017

DISCUSSION

Melanoma is one of the most frightful human diseases in both developed and developing countries, largely due to its high-grade malignancy, fast infiltrating growth, and early metastasis[21]. Early detection of melanoma can contribute to a favorable prognosis and survival rate for melanoma patients. Unfortunately, current diagnostic methods are limited, so it is urgently needed to explore new sensitive and cost-effective biomarkers for the development of novel noninvasive tools[22]. Recently, more and more studies have suggested many biomarkers for the prognosis and diagnosis of melanoma patients, such as specific expression genes, methylation levels, serum miRNAs, and lncRNAs[23,24]. Among them, lncRNAs attract growing attention due to their high specificity, high sensitivity, and noninvasive characteristics.

Recent studies have demonstrated that several lncRNAs participate in the initiation and progression of melanoma via modulating important tumor-associated genes and various miRNAs[25]. Wang et al.[26] suggested lncRNA CASC2 as a tumor suppressor due to its significant downregulation in melanoma patients and its overexpression suppressing the proliferation and metastasis of melanoma cells via sponging miR-181a. A study from Jiao et al.[27] showed that lncRNA LINC00963, an overexpressed lncRNA in melanoma, exhibited a tumor-promotive role by inhibiting the metastasis potential of melanoma cells via sponging miRNA-608 to increase NACC1 expression. Besides, several lncRNAs have been confirmed to serve as tumor biomarkers for the diagnosis and prognosis of melanoma patients[28]. These findings encouraged us to further explore more tumor-related lncRNAs. Recently, the distinct upregulation of LINC00173 was reported in several tumors and its tumor-promotive role was also confirmed in breast cancer and lung cancer[17,19]. However, the expression and clinical significance of LINC00173 in melanoma have not been investigated.

In this study, we identified a novel melanoma-related lncRNA, i.e., LINC00173, which was confirmed to be distinctly overexpressed in our cohort (163 patients). Also, we observed that the patients with advanced stages displayed a higher level of LINC00173, indicating that LINC00173 may contribute to the clinical progression of melanoma. Then, we performed ROC assays for the determination of the diagnostic value of LINC00173 expression, finding that high LINC00173 levels were robust in distinguishing melanoma tissues from

normal melanoma specimens. In addition, the similar results were also observed when we further performed subgroup analysis based on the clinical stages of 163 melanoma patients. Moreover, clinical assays revealed that melanoma patients with higher LINC00173 expressions were associated with lymph node metastasis and advanced tumor stage, and suffered poorer OS and DFS, suggesting it may act as a tumor promotor in the clinical progress of melanoma patients. Finally, multivariate assays demonstrated high LINC00173 expression as an independent indicator of unfavorable OS and DFS of melanoma patients. Our findings may help the development of novel clinical tools in the early screening and the prediction of clinical prognosis before the clinical treatments.

Several limitations should be considered. First, the sample size was relatively small, and large clinical trials were needed to conduct. Second, replications in other groups were not conducted. Finally, the potential function and molecular mechanisms of LINC00173 were not explored in melanoma.

CONCLUSION

Our study presented that LINC00173 expression was abnormally elevated in melanoma and may serve as a novel biomarker for predicting diagnosis and clinical progression of melanoma patients.

AUTHORS' CONTRIBUTIONS

MW, WL: Writing – original draft, Data curation, Formal Analysis. **WL:** Conceptualization, Data curation. **CW:** Conceptualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Cardiovascular drugs and analysis of potential risk factors associated with mortality in severe coronavirus disease 2019 patients

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SUMMARY

OBJECTIVES: Cardiovascular diseases are also considered to increase the risk of death in COVID-19 patients. However, real-world data concerning the risk factors for death in patients with severe COVID-19 still remain vague. This study aimed to identify the potential risk factors associated with mortality in severe COVID-19 patients.

METHODS: All consecutive patients admitted to the intensive care unit (ICU) of our institute for COVID-19 for severe COVID-19 pneumonia from April 1, 2020 to July 20, 2020 were included in the analysis. Patient characteristics, including complete medical history and comorbid diseases, blood test results during admission and on day 7, and clinical characteristics were compared between survivors and nonsurvivors.

RESULTS: There was no significant difference between survivors and nonsurvivors regarding age, gender, and preexisting cardiovascular diseases. Moreover, the rate of the medications including angiotensin-converting enzyme (ACE) inhibitor and angiotensin receptor blockers did not differ between survivors and nonsurvivors. The peak C-reactive protein (CRP), procalcitonin, fibrinogen, and D-dimer levels and the rate for chronic renal failure were significantly higher in nonsurvivors compared with survivors. Intubated patients had a higher risk of death than the others had.

CONCLUSIONS: This study failed to demonstrate a significant difference in preexisting cardiovascular diseases and cardiovascular medications between survivors and nonsurvivors who were admitted to ICU for severe COVID-19. Our findings indicate that the presence of chronic renal failure, a high peak ferritin concentration, and the need for invasive mechanical ventilation appear predictive for mortality. We propose that these risk factors should be taken into account in defining the risk status of severe COVID-19 patients admitted to the ICU.

KEYWORDS: COVID-19. Cardiovascular drugs. Potential risk factors. Renal failure. Intubation.

INTRODUCTION

A few cases of acute respiratory distress syndrome (ARDS) accompanying pneumonia with unknown etiology have been observed in Wuhan, China in late 2019[1]. Although the course of the disease was favorable in some of the cases, noninvasive and invasive mechanical ventilations were required in serious cases in whom fever and fatigue were the predominant symptoms[2]. A novel coronavirus, named 2019-nCoV by the World Health Organization (WHO), was identified in the throat swab samples of subjects[3]. After the rapid spread of the confirmed cases, it was defined as the outbreak by the WHO. Then, the International Committee on Taxonomy of Viruses decided to rename the virus as the SARS-CoV-2[4]. Since around October 3, 2020, it has been recorded that more than 35 million people were infected and 1 million deaths were recorded worldwide due to COVID-19[5]. The lung is the main target organ for COVID-19 and causes pneumonia; however, novel data indicate that COVID-19 is a systemic disease, which presents with gastrointestinal and neurological disorders, renal failure, and some cardiologic and pulmonary problems[6]. Certain populations including immunocompromised individuals, those with pulmonary disease, diabetes, and being above 65 years are regarded as the higher risk group during COVID-19[7]. Cardiovascular diseases are also considered to raise the mortality risk in COVID-19 cases[8]. However, real-world data about the risk factors for mortality in COVID-19 still remain unproved.

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In this study, we aimed to find out the possible risk factors related to mortality and the impact of preexisting cardiovascular diseases and medications on mortality in hospitalized COVID-19 cases.

METHODS

This cohort study was comprised of the patients admitted to the intensive care unit (ICU) for severe COVID-19 pneumonia from April 1, 2020 to July 20, 2020, who were included in retrospective analysis. Demographic and clinical characteristics, comorbid diseases, and daily blood test results were recorded. COVID-19 was diagnosed with the polymerase chain reaction (PCR) test in patients presenting with flu-like symptoms. All patients signed informed consent. The local ethical committee approved the study, and necessary procedures were followed up.

Severe COVID-19 pneumonia was defined as $SatO_2$<90% or PaO_2<70 mmHg, PaO_2<70 mmHg despite 5 L/min nasal O_2 supplementation, a respiratory rate>30/min, the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2)<300, lactate>4 mmol/L or lung infiltrates>50%. Patients were treated with favipiravir 1600 mg twice daily on the 1st day of treatment and 600 mg twice daily for 4 days, azithromycin 250 mg once daily following a 500-mg loading dose, a prophylactic dose of low-molecular-weight heparin based on the patient's weight, and corticosteroids (dexamethasone and methylprednisolone) based on the patient's symptoms. Patients admitted to ICU were also treated similarly. The hospital digital database was used for the patients' records. The study group was divided into two groups based on the survival status, namely, survivors and nonsurvivors.

Statistical analysis

Data were recorded and analyzed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). p<0.05 was accepted as the statistically significant level. Normality was tested using Q-Q plots. Mean±standard deviation (SD) or median (1st–3rd quartile) were used to express continuous data according to the normality level. Normally distributed data were compared using t-test, while nonnormally distributed data were analyzed using the Mann–Whitney U-test. The chi-square test was used for the categorical data comparison. Paired continuous variables were compared using the analysis of variance (ANOVA) and Wilcoxon signed-rank test.

RESULTS

A total of 200 COVID-19 patients were admitted to ICU in a defined time period for this study [median age 68 (56.5–76) years, 55.5% male]. Notably, 97 of them were discharged, while 103 of them died during hospitalization. Nonsurvivors were older [70 (59–78) years vs. 66 (53–74), years, p=0.008] and had a higher rate for chronic renal failure compared with survivors (39.81% vs. 15.46%, p<0.001). Both groups were similar with respect to gender, age, and preexisting other diseases. Moreover, the rate of the medications used for comorbid diseases was also similar for both groups (Figure 1A). However, the intubation rate was found to be greater in nonsurvivors compared with survivors (Figure 1B).

The comparison and distribution of measurements of the patients are summarized in Table 1. A significant increase was observed in the leukocyte count (p=0.001), procalcitonin (p<0.001), aPTT (p=0.029), fibrinogen (p=0.007), AST and ALT (p<0.001), mean platelet volume (p<0.001), and ferritin concentration (p=0.001) and in glomerular filtration rate (p=0.001) from baseline to day 7 of COVID-19 among non-survivors. In contrast, lymphocyte (p=0.015), monocyte count (p = 0.009) and albumin concentration (p < 0.001) decreased significantly from baseline to day 7 of COVID-19 among nonsurvivors.

To evaluate the independently related factors with mortality, we conducted multiple logistic regression analysis (Table 2). We detected that patients who needed intubation had a 232.669-fold higher risk of mortality compared with patients without intubation [odds ratio (OR): 232.669, 95% confidence interval (CI): 50.198–1078.429, and p<0.001)]. The renal failure increased the risk of mortality 3.524-fold (OR: 3.524, 95%CI: 1.133–10.956, and p=0.030). Moreover, we also noticed that higher ferritin rates (p=0.035) are associated with an elevated risk of mortality (Figure 1C and D).

DISCUSSION

This study shows that the rate of concomitant cardiovascular disease and medications used for preexisting cardiovascular disorders do not differ between survivors and nonsurvivors from severe COVID-19. However, chronic renal failure is more prevalent among nonsurvivors relative to survivors. Our findings indicate that presence of chronic renal failure, a high peak ferritin concentration, and the need for invasive mechanical ventilation appear predictive for mortality.

The mortality from COVID-19 is continuing to rise globally. Identifying the factors associated with mortality, particularly in those with severe illness, is therefore critical to introduce advanced treatment options and to forecast who will need ICU admission. Early reports from China have revealed that age, elevation in inflammatory markers, and the presence

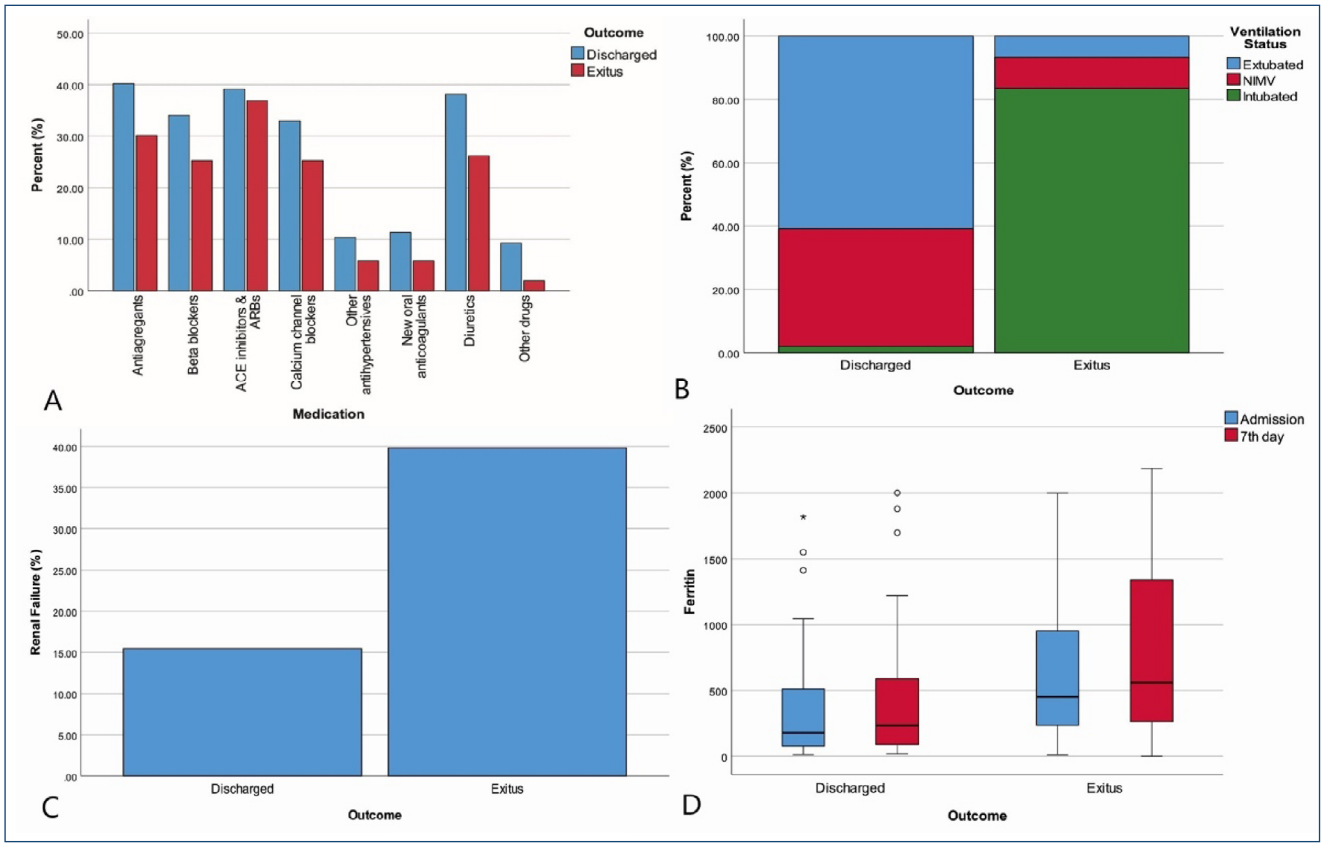

Figure 1. Results of **(A)** history of use of cardiovascular medications, **(B)** ventilation status, **(C)** renal failure percentages, and **(D)** ferritin values. * indicates that the p-value is statistically significant but close to each other.

Table 1. Summary of biochemical measurements during admission and on day 7.

	Outcome		Total	p (between variables)
	Discharged (n=97)	Exitus (n=103)		
WBC				
Admission	8.3 (6.2–12)	10.5 (7.2–15)	9.56 (6.45–13.45)	**0.012**
Day 7	8.3 (6–12)	13 (9.5–17)	10.6 (7.23–14.8)	**<0.001**
p (within variables)	0.575	**0.001**	**0.012**	
CRP				
Admission	44 (11.8–124.7)	93 (39–157)	73.5 (19.8–141.5)	**0.005**
Day 7	16 (7.8–68)	94 (34–154)	50 (10.83–112)	**<0.001**
p (within variables)	**<0.001**	0.745	**0.002**	
Procalcitonin				
Admission	0.19 (0.09–0.79)	0.60 (0.19–2.00)	0.30 (0.12–1.30)	**<0.001**
Day 7	0.12 (0.05–0.45)	1.90 (0.50–5.30)	0.49 (0.09–2.20)	**<0.001**
p (within variables)	**<0.001**	**<0.001**	0.053	
PT				
Admission	72.53±18.67	71.77±18.92	72.14±18.76	0.776
Day 7	75.90±17.23	67.13±18.64	71.38±18.45	**0.001**
p (within variables)	0.066	**0.009**	0.618	

Continue...

Table 1. Continuation.

	Outcome		Total	p (between variables)
	Discharged (n=97)	Exitus (n=103)		
aPTT				
Admission	31 (27–35)	29 (24.6–34)	30 (26–35)	0.149
Day 7	30 (26–35)	31 (28–37)	30 (27–35.5)	0.111
p (within variables)	0.335	**0.029**	0.346	
INR				
Admission	1.10 (1.00–1.23)	1.10 (1.00–1.30)	1.10 (1.00–1.30)	0.343
Day 7	1.09 (1.00–1.20)	1.20 (1.00–1.40)	1.10 (1.00–1.30)	**0.003**
p (within variables)	0.074	0.133	0.858	
Fibrinogen				
Admission	381.89±142.44	458.81±146.93	421.50±149.46	**<0.001**
Day 7	377.31±139.45	488.58±160.81	434.61±160.45	**<0.001**
p (within variables)	0.687	**0.007**	0.112	
D-Dimer				
Admission	413 (178–723)	1155 (598–6765)	673 (339.5–2196)	**<0.001**
Day 7	410 (188–996)	1723 (650–4580)	850.5 (336.5–2373.5)	**<0.001**
p (within variables)	0.363	0.704	0.503	
Fasting blood glucose				
Admission	135 (106.9–174)	164 (118–225)	148.5 (114.5–205.5)	**0.024**
Day 7	124 (101–176)	169 (115–271)	145 (105–225)	**0.002**
p (within variables)	0.385	0.327	0.910	
AST				
Admission	27 (18–41)	36 (22–61)	29.5 (20.35–49.5)	**0.008**
Day 7	22 (17–33)	61 (32–150)	33 (19–79)	**<0.001**
p (within variables)	0.091	**<0.001**	**0.012**	
ALT				
Admission	21 (14–40)	23 (13–40)	22 (13.5–40)	0.487
Day 7	24 (17–35)	43 (21–80)	30 (19–56.25)	**<0.001**
p (within variables)	0.117	**<0.001**	**<0.001**	
CK				
Admission	90 (57–202)	147 (55–330)	107.5 (56–242.5)	**0.014**
Day 7	51 (34–109)	185 (66–433)	93 (41.5–287)	**<0.001**
p (within variables)	**<0.001**	0.154	0.233	
CK-MB				
Admission	29 (19–47)	39 (25–65)	34 (21–58)	**<0.001**
Day 7	24 (15–35)	40 (25–70)	32.9 (19.5–53.5)	**<0.001**
p (within variables)	0.086	0.831	0.198	
Troponin				
Admission	0.1 (0.1–0.1)	0.1 (0.1–0.32)	0.1 (0.1–0.16)	0.059
Day 7	0.1 (0.1–0.1)	0.1 (0.1–1.5)	0.1 (0.1–0.43)	**<0.001**
p (within variables)	**0.025**	0.662	0.483	

Continue...

Table 1. Continuation.

	Outcome		Total	p (between variables)
	Discharged (n=97)	Exitus (n=103)		
GFR				
Admission	75 (41–94)	53 (38–77)	62 (39–87.25)	**0.003**
Day 7	77 (51–102)	44 (17–71)	60 (29.5–89.5)	**<0.001**
p (within variables)	**0.002**	**0.001**	0.621	
Lymphocyte				
Admission	9.8 (5.7–20)	6.7 (3.2–12.6)	7.75 (4.4–16.25)	**0.001**
Day 7	12 (6.6–20)	5.5 (3.4–9.6)	7.95 (4.2–14.95)	**<0.001**
p (within variables)	0.313	**0.015**	0.298	
Monocyte				
Admission	5.2 (3.7–7.1)	4.2 (2–6)	4.8 (2.85–6.4)	**0.004**
Day 7	5.9 (4–7.1)	2.8 (2–5.2)	4.4 (2.55–6.4)	**<0.001**
p (within variables)	0.613	**0.009**	0.149	
MPV				
Admission	10.38±1.25	9.80±1.10	10.08±1.21	**0.001**
Day 7	9.47±1.11	11.50±1.65	10.52±1.74	**<0.001**
p (within variables)	**<0.001**	**<0.001**	**<0.001**	
Ferritin				
Admission	178 (76–510)	450 (235–963)	342 (107.5–713.5)	**<0.001**
Day 7	235 (88–588)	560 (257–1353)	365.5 (131–880.5)	**<0.001**
p (within variables)	**0.004**	**0.001**	**<0.001**	
Albumin				
Admission	3.44±0.44	3.35±0.36	3.39±0.41	0.102
Day 7	3.27±0.46	2.45±0.38	2.85±0.59	**<0.001**
p (within variables)	**<0.001**	**<0.001**	**<0.001**	

Data are given as mean±standard deviation or median (1st–3rd quartile) according to the normality of distribution. Bold values denote statistical significance at p<0.05.

Table 2. Significant risk factors of the death and multiple logistic regression analysis.

	β	Standard error	Wald	p	Exp (β)	95% Confidence interval for Exp (β)	
Renal failure	1.260	0.579	4.737	0.030	3.524	1.133	10.956
Intubated	5.450	0.782	48.504	<0.001	232.669	50.198	1078.429
Ferritin	0.001	0.001	4.435	0.035	1.001	1.000	1.002
Constant	-2.571	0.449	32.787	<0.001	0.076		

Dependent variable: outcome (Exitus); Nagelkerke R^2=0.768.

of underlying diseases or secondary infection were predictive for fatal outcome[9]. Several reviews and meta-analysis investigating the risk factors for mortality have been published recently. Subjects with concomitant cardiometabolic disease and those presenting with acute inflammation and end-organ damage have been shown to have a higher risk of death from COVID-19[10]. In-hospital mortality due to COVID-19 was reported to be 4.85 fold increased in patients with cardiovascular disorders, particularly in those with elevated troponin levels[11]. A recent prospective cohort study has reported that

the presence of cardiovascular or cerebrovascular diseases, age of ³65 years, and cardiac troponin I higher than 0.05 ng/mL were correlated with high mortality rate in COVD-19 patients[12]. Elevated neutrophil-to-lymphocyte ratio, D-dimer, C-reactive protein (CRP), aspartate aminotransferase, and reduced albumin concentration and glomerular filtration rate were also found to be related to a bad outcome in COVID-19 patients[13]. The meta-analysis that included 21 papers including 3,377 patients has reported that compared with nonserious diseases and survivors, subjects with severe and terminal diseases had significantly higher leukocyte, ferritin, and lymphopenia counts. Unlike the group of survivors, heart attack, surrogate markers of inflammation, liver and kidney functions, and coagulopathy have been observed to be elevated in nonsurvivors[14].

In this study, we indicated that there is no impact of comorbid cardiovascular diseases on mortality in severe COVID-19. This is somewhat challenging with the results of previous data. We speculate that the severity of the preexisting cardiovascular disease has a higher impact on mortality than the sole presence of concomitant cardiovascular disease in COVID-19. In other words, subjects with severe left ventricular dysfunction might have poorer outcomes from COVID-19 than those with mild left ventricular systolic dysfunction, although both are classified as heart failure. In addition, subjects with severe multivessel coronary artery disease might have a higher mortality from COVID-19 than those with single-vessel coronary artery disease, although both are defined as atherosclerotic cardiovascular disease. From this point of view, we considered that clinicians should take the severity of preexisting cardiovascular disorders into account to address the underlying disease as a significant predictor of mortality from COVID-19.

Findings of this study have also shown that the use of preexisting medications, including calcium channel blockers, antiaggregant, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers, beta-blockers, and oral anticoagulant agents used for comorbid disease, was similar between survivors and nonsurvivors. This finding supports previous evidence indicating that ACE inhibitors and angiotensin receptor blockers have no significant impact on mortality in COVID-19[15,16]. We consider that ACE inhibitors and angiotensin receptor blockers should be continued even in severe COVID-19 patients since treatment with these agents appears safe.

This study has also demonstrated that CRP, procalcitonin, and fibrinogen, which are surrogates of inflammatory state, and D-dimer level, which is the indicator of a prothrombotic state, have been found elevated in the group of nonsurvivors related to the other group from severe COVID-19. These results are compatible with the data gathered from the previous studies[17]. The recent information indicates that acquired, endothelial interaction-mediated prothrombotic coagulopathy had a role in worsening clinical course[18]. Accordingly, elevated D-dimer levels found among nonsurvivors in our research can be explained by the existence of a prothrombotic environment secondary to serious COVID-19.

Logistic regression analyses showed that intubation and high ferritin concentration were independent predictors for mortality in severe COID-19 patients. Patients requiring intubation and mechanical ventilation have been noted to have advanced pulmonary involvement; thus, these patients are at high risk for mortality[19]. The increase in ferritin concentrations among no-survivors is probably associated with the hyperinflammation-cytokine storm which leads to fatal multi-organ failure[20]. We also found that chronic kidney disease was an independent indicator for death in COVID-19. The early data regarding the role of renal failure on mortality in COVID-19 support our finding. Cheng et al[21]. have reported that the prevalence of kidney disease on admission was correlated with in-hospital death rates in COVID-19[21].

Accordingly, we consider that subjects with severe COVID-19, increased CRP, procalcitonin, fibrinogen, D-dimer levels, and chronic renal disease are at greater risk for mortality. Thus, these subjects might be candidates for corticosteroids and IL-6 antagonists, which have been shown to improve survival in critically ill COVID-19 patients.

The major limitation of this research is the comparatively limited sample size. The other one is also the absence of details about the level of cardiovascular comorbidity. We did not investigate the duration of disease, functionality levels, and other parameters which could be the important factors for the prognosis of cardiovascular patients.

CONCLUSIONS

This study failed to demonstrate the significant difference in preexisting cardiovascular diseases and cardiovascular drugs between survivor and nonsurvivor patients who were admitted to ICU for severe COVID-19. However, CRP, ferritin, procalcitonin, fibrinogen, and D-dimer levels in nonsurvivors were higher than that of survivors indicating that the hyperinflammation and prothrombotic state may have a role on poor outcomes in COVID-19 patients. Our findings indicate that presence of chronic renal failure, a high peak ferritin concentration, and the need for invasive mechanical ventilation appear predictive for

mortality. We propose that these risk factors should be taken into account in defining the risk status of severe COVID-19 patients admitted to the ICU.

ETHICAL APPROVAL

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics for the study were obtained from the Van Training and Research Hospital (dated: 08/07/2020 decision number: 2020/13). Informed consent was obtained from all individual participants included in this study.

AUTHORS' CONTRIBUTIONS

RDA, NA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **AŞ, TA:** Project administration, Resources, Software, Supervision. **ZD, MÖ:** Data curation, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Can 1st and 6th month pulmonary function test follow-ups give an idea about the long-term respiratory effects of COVID-19 pneumonia?

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SUMMARY

OBJECTIVE: The aim of this study was to ascertain the long-term respiratory effects of COVID-19 pneumonia through pulmonary function tests in follow-ups at 1 and 6 months.

METHODS: Our study was conducted between August 1, 2020 and April 30, 2021. At 1 month after discharge, follow-up evaluations, PFTs, and lung imaging were performed on patients aged above 18 years who had been diagnosed with COVID-19 pneumonia. In the 6th month, the PFTs were repeated for those with pulmonary dysfunction.

RESULTS: A total of 219 patients (mean age, 49±11.9 years) were included. Pathological PFT results were noted in the 1st month for 80 patients and in the 6th month for 46 (7 had obstructive disorder, 15 had restrictive disorder, and 28 had small airway obstruction) patients. A significant difference was found between abnormal PFT results and patient-described dyspnea in the 1st month of follow-up. The 6-month PFT values (especially those for forced vital capacity) were statistically significantly lower in the patients for whom imaging did not indicate complete radiological improvement at the 1-month follow-up. No statistically significant difference was found between the severity of the first computed tomography findings or clinical condition on emergency admission and pulmonary dysfunction (Pearson's chi-square test, P=0.904; Fisher's exact test, P=0.727).

CONCLUSION: It is important that patients with COVID-19 pneumonia be followed up for at least 1 month after discharge to be monitored for potential long-term lung damage. PFTs should be administered to those in whom ongoing dyspnea, which started with COVID-19, and/or full recovery were not identified in pulmonary imaging.

Keywords: Respiratory function tests. COVID-19. Lung injury. Lung diseases. Long term adverse effects.

INTRODUCTION

The long-term damage to patients with coronavirus disease 2019 (COVID-19) is slowly being revealed. It is clear that more time will be required for the completion of studies on this issue. The effects are not limited to the respiratory system. Almost all body systems are involved[1]. Individual characteristics might play a role. Many conditions, such as advanced age, diabetes, hypertension, obesity, and coronary artery disease history, are the causes of poor clinical outcomes related to respiratory tract infections during the acute period[2].

In a recent study, Anastasio et al. reported a correlation between COVID-19-related lung damage and reduced pulmonary function 4 months after acute infection[3]. The patients' recovery process should be carefully observed to prevent possible long-term problems and to establish treatment modifications. Therefore, in this study, it was aimed to investigate the usability of pulmonary function tests (PFTs) at 1- and 6-month follow-ups for early diagnosis in the development of long-term respiratory effects of COVID-19 pneumonia. The determination of the influential variables in the development of permanent lung damage was the secondary goal.

METHODS

Ethical statement

This study was approved by the local institutional review board (Approval No. B.10.1.TKH.4.34.H.GP.0.01/170). It was conducted in compliance with the World Medical Association Declaration of Helsinki. In addition, written informed consent was obtained from the participants.

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Study design and population

This single-center prospective observational study was conducted at University of Health Sciences Umraniye Training and Research Hospital between August 1, 2020 and April 30, 2021. During the first 3 months of this period, the study patients were determined, and during the succeeding 6 months, 1- and 6-month follow-up evaluations, which included PFTs, were performed. The inclusion criteria for the study were as follows: (a) application to the emergency department; (b) receipt of positive reverse transcription-polymerase chain reaction results; (c) clinico-radiological diagnosis of COVID-19 pneumonia; (d) age >18 years; (e) absence of abnormalities, such as lung disease, upper respiratory tract obstruction, neuromuscular disease, kyphoscoliosis, and ankylosing spondylitis, which could affect the PFT values; (f) absence of diseases, such as neuropsychiatric diseases, facial paralysis, mental retardation, and dementia, which could affect cooperation during PFT; and (g) presentation of the patient-signed informed consent form. Patients who did not meet these criteria and those whose records were incomplete were excluded from this study.

Patients who met the inclusion criteria were invited for a follow-up at the end of the 1st month after inpatient or outpatient COVID-19 pneumonia treatment. During the follow-ups, the status of COVID-19 pneumonia respiratory tract complaints was questioned, and the presence of ongoing symptoms was investigated. In addition, the Modified Medical Research Council (mMRC) scale, Borg rating of perceived exertion scale, control blood tests, PFTs, and pulmonary imaging (chest X-rays and/or pulmonary CT, if necessary) were administered. The evaluations of CT findings were based on the current literature[4]. We terminated the patients' follow-up whose respiratory evaluations were normal during the follow-up period and the collection of their data. Those whose respiratory evaluations indicated a lack of improvement were recalled for a 6-month follow-up, which included a PFT. Pulmonary imaging was repeated for the patients in whom radiological abnormalities were identified in the first control examination at the 6-month follow-up. Invitations to participate in follow-ups were offered face-to-face at discharge or by telephone later. In addition, the patients received a reminder telephone call during the week preceding the follow-up date. Patients who did not come to the follow-up and those who wanted to leave the study were excluded. The PFTs were performed by professionally trained respiratory technicians and interpreted by a pulmonologist with 20 years of experience. The final medical management decisions related to the post-discharge evaluations were made by the same pulmonologist.

Data collection

Data on demographics and comorbidities, clinical and vital parameters, and laboratory, radiodiagnostic, and lung function tests were obtained through real-time patient examinations and the patient records in the hospital data management system. The results of the mMRC and Borg scale, which were administered to evaluate dyspnea severity during the 1-month follow-up, were also recorded. During the telephone calls, the researchers recorded identifiable changes in the patients' health status. A Spirolab III spirometer (Medical International Research, Rome, Italy) was used for the PFTs. Several other parameters, such as FVC, forced expiratory volume in one second (FEV1), FEV1/FVC, peak expiratory flow, and forced expiratory flow (normal range 25–75%), were evaluated. Microsoft Excel® (Version 2019 for Windows, Microsoft Corp.) was used to record the patient data throughout the study period.

Statistical analysis

The statistical tests were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Conformity of the continuous data to normal distribution was determined using the Shapiro–Wilk test. The Student's *t*-test was used for intergroup comparisons of the normally distributed continuous data, and the Mann–Whitney *U*-test was used for the nonnormally distributed continuous data. The chi-square test was used for the comparisons of the categorical data. Fisher's exact test was used where necessary. The mortality-related variables were determined by logistic regression. The variables that were determined to be statistically significant by the univariate analysis and the clinically significant variables with a p-value <0.2 were included. Statistical significance was set as $P<0.05$.

RESULTS

The study included 219 patients (mean age, 49±11.9 years), 39.7% of whom were women (Table 1). It was determined that none of these patients died during the study period. PFTs were performed on all patients in the 1st month after treatment. Pathology was detected in 80 (36.5%) patients. In the 6th month, control PFTs were performed on these patients. Pulmonary dysfunction was found in 46 (21% of all patients) patients (Table 2).

At the 1-month follow-up, the patients were asked about respiratory distress. Of note, 178 patients did not experience respiratory distress. However, 41 patients did not have fully improved symptoms. They had either effort-related or noneffort-related respiratory distress. A comparison of the PFT values in the groups with and without respiratory distress indicated

Table 1. Basic characteristics of the study population.

	N (%)/Mean ±SD/Median (25–75%)
Age, years	49±11.9
Sex (female)	87 (39.7)
Background	
Diabetes mellitus	27 (12.3)
Hypertension	40 (18.3)
Chronic renal failure	5 (2.3)
Coronary artery disease	11 (5)
Congestive heart failure	3 (1.4)
Thyroid disorder	7 (3.2)
Active smoking	16 (7.3)
Pack/year ratio in smoking patients	14 (5–20)
Occupational exposure	7 (3.2)
Body mass index	
<25	49 (22.4)
25–30	95 (43.4)
>30	74 (33.8)
Complaint	
Fever	93 (42.5)
Coughing	87 (39.7)
Dyspnea	34 (15.5)
Fatigue	97 (44.3)
Hemoptysis	1 (0.5)
Other symptoms	123 (56.2)
Asymptomatic patients	8 (3.7)
Hospital admission	
Outpatient treatment	26 (11.9)
Ward admission	173 (79)
ICU admission	9 (4.1)
Hospital length of stay (days)	6 (5–10)
Total time of treatment	7.5 (5–14)
Initial computed tomography findings	
Patients underwent thoracic tomography at first admission	209 (95.4)
Presence of any pathological finding	204 (93.2)
Findings of preexisting lung disease	4 (1.8)
Typical COVID-19	180 (82.2)
Atypical COVID-19 findings	19 (8.7)

Continue...

Table 1. Continuation.

	N (%)/Mean ±SD/Median (25–75%)
Ground-glass opacification	19 (8.7)
Consolidation	113 (51.6)
Bilateral involvement	164 (74.9)
Presence of nodules	17 (7.8)
Pleural effusion	0 (0)
Thoracic lymphadenopathy	1 (0.5)
Other (calcification, cavitation, bronchiectasis, etc.)	17 (7.8)
Disease severity in terms of CT distribution	
No distribution	7 (3.2)
Mild distribution	121 (55.3)
Moderate distribution	60 (27.4)
Severe distribution	21 (9.6)
mMRC score	0 (0–0)
Borg score	0 (0–1)
Pulmonary function test results at the 1st month follow-up	
FVC, lt (for all patients)	3.28 (2.83–4.06)
FVC, % (for all patients)	88 (77.5–100)
FEV1, L (for all patients)	3.13 (2.47–3.67)
FEV1, % (for all patients)	96.7±18.1
FEV1/FVC, % (for all patients)	92.4 (87.6–96.3)
PEF, lt (for 209 patients)	6.18 (4.76–8.54)
PEF, % (for 209 patients)	81 (62–101.5)
FEF 25–75, lt (for 209 patients)	3.92 (3.4–4.9)
FEF 25–75, % (for 209 patients)	105 (87.5–125)
Obstructive pathology**	
None	39 (17.8)
Mild	6 (2.7)
Severe	1 (0.5)
Restrictive pathology**	
None	29 (13.2)
Mild	14 (6.4)
Moderate	1 (0.5)
Severe	0
Patients with small airway**	28 (12.8)
Number of patients who underwent PFT at both 1 and 6 months	80 (36.5)

Continue...

Table 1. Continuation.

	N (%)/Mean ±SD/Median (25–75%)	
Vital parameters	**Initial admission**	**Control admission**
Heart rate, beats per minute	85.5 (78–92.75)	89 (81–99.75)
Systolic blood pressure, mmHg	115 (109–120)	–
Diastolic blood pressure, mmHg	70 (67.3–80)	–
spO_2, %	96.5 (94.3–98)	98 (97–98)
Laboratory results		
Lymphocyte, $10^3/\mu L$	1.5 (1.13–2.02)	2.14 (1.82–2.61)
CRP, mg/L	1.2 (0.2–4)	0.2 (0.2–0.3)
Elevated liver enzymes	12 (5.5)	7 (3.2)
Impaired kidney function tests	4 (1.8)	4 (1.8)

*Those who had never smoked or quit for more than 1 year were considered nonsmokers. **Patients who still have pathological findings in the second pulmonary function test (PFT) results 6 months later.

statistically significantly lower PFT values in the patients who described respiratory distress (Table 2). The 6-month control PFTs revealed obstructive lung disease in 7 patients, restrictive lung disease in 15, and small-airway obstruction in 28.

The patients were clinically divided into *severe COVID-19* and *other* (mild–moderate) groups. In the 1st month, PFT abnormalities were observed in four (44.4%) of the nine patients in the severe COVID-19 group and 76 (36.2%) of the 210 patients in the other group. There was no statistically significant difference between the groups (Fisher's exact test, P=0.727).

When necessary, chest X-rays and/or CT imaging of the thorax were performed at the 1-month follow-up. The results indicated that 169 (77.2%) had complete radiological recovery; however, 38 had not completely recovered. In our study, the control CT imaging results were compared with the PFT results of the patient groups at the 1st month follow-up. A statistically significant decrease was found in the PFT values, especially those for FVC, in the patients with radiological improvement (Table 2).

According to the findings of the first CT (no lung involvement and mild, moderate, and severe lung involvement) performed during emergency admission, the frequencies regarding pathology in the 1-month PFT results were 2 (28.6%), 43 (35.5%), 24 (40%), and 8 (38.1), respectively. However, no statistically significant difference was found between the groups (chi-square test, P=0.904).

Table 2. Comparison of different patient groups according to their pulmonary function tests results at follow-ups.

	Median (25–75%)		p-value
Comparison of 1st month and 6th month PFT results in patients with detected PFT pathology in the 1st month (n=80).			
	1st month	**6th month**	
FVC, lt *	3.35 (2.92–3.93)	3.39 (2.74–4.13)	0.646
FVC, % *	88 (75.75–99.5)	84 (73–100)	0.161
FEV1, lt *	3.13 (2.57–3.52)	2.89 (2.38–3.58)	**0.025**
FEV1, % *	95 (84–110.25)	89 (80–103)	**<0.001**
FEV1/FVC, % *	92.90 (88.58–96.08)	89.3 (85.7–95.1)	**0.003**
PEF, lt **	5.73 (4.77–8.88)	4.89 (3.71–6.38)	**<0.001**
PEF, % **	78 (61–126)	60 (47–78)	**<0.001**
FEF 25–75, lt **	3.94 (3.43–5.13)	3.35 (2.67–4.64)	**<0.001**
FEF 25–75, % **	105 (91.5–129)	96 (71–114)	**<0.001**
Comparison of patient groups with and without dyspnea in terms of PFT results at the 1st month follow-up.			
	Dyspnea (–)	**Dyspnea (+)**	
FVC, lt *	3.39 (2.93–4.13)	2.84 (2.32–3.63)	**0.001**
FVC, % *	89 (79–101)	81.5 (68.5–95)	**0.027**
FEV1, lt *	3.13 (2.55–3.74)	2.75 (2.22–3.28)	**0.009**
FEV1, % *	97 (86–109)	91.5 (77.5–106.75)	**0.040**
FEV1/FVC, % *	91.5 (86.8–96.2)	95.05 (91.43–100)	**0.004**
PEF, lt **	6.53 (4.97–8.82)	5.14 (4.34–7.33)	**0.024**
PEF, % **	83 (64–103)	71.3 (57.25–95.25)	0.137
FEF 25–75, lt **	3.93 (3.38–5.13)	3.86 (3.43–4.55)	0.445
FEF 25–75, % **	106 (85–125)	104 (91–125.25)	0.962
Comparison of PFT results of the patient groups with and without complete radiological improvement at the 1st month follow-up.			
	Radiological improvement (–)	**Radiological improvement (+)**	
FVC, lt *	3.13 (2.3–3.83)	3,32 (2.92–4.13)	**0.033**
FVC, % *	81 (71.5–94)	89 (81–89)	**0.041**
FEV1, lt *	2.85 (2.15–3.52)	3.13 (2.52–3.68)	0.112
FEV1, % *	89 (80.5–107.5)	98 (87–109)	0.051
FEV1/FVC, % *	93.2 (90.5–97)	91.7 (86.5–96.15)	0.095
PEF, lt **	6.67 (4.98–9.15)	6.17 (4.73–8.36)	0.630
PEF, % **	91 (63.5–105)	78.5 (61–100.75)	0.266
FEF 25–75, lt **	3.88 (3.42–5.38)	3.93 (3.38–4.76)	0.787
FEF 25–75, % **	111 (93.5–133.5)	105 (87–122)	0.123

*These values are for all patients for whom 1st and 6th month PFT results are available (80 patients). **These values are the data of 78 patients who were able to comply with the test performed. Bold values indicate statistically significance.

The 1-month PFT results in terms of the mMRC and Borg scale results indicated that there was no statistically significant difference between the patients with pathology and those without (Mann–Whitney *U*-test: P=0.986 and 0.820, respectively). In addition, no statistically significant difference was found between the groups with and without pathology in the 6-month follow-up PFT results in terms of the mMRC and Borg scale results that were applied during the 1st month (Mann–Whitney *U*-test: P=0.795, P=0.611, respectively).

When the patients were dichotomized into the severe COVID-19 and other groups on the basis of their initial CT results, the median mMRC score of those with severe lung involvement was 0 (0–0). For those in the other group, it was 0 (0–1). There was no statistically significant difference between the groups (Mann–Whitney *U*-test, P=0.075). The median Borg scale scores were 0 (0–1) for the patients with severe COVID-19 and 0 (0–1.5) for the other patients. No statistically significant difference was found between the groups (Mann–Whitney *U*-test, P=0.314).

There was no statistically significant difference in the mMRC scores of the obstructive lung disease groups (mild, moderate, and severe), which were established on the basis of the PFT results (Kruskal–Wallis test, P=0.892). Again, no statistically significant difference was found in the scores of the restrictive lung disease groups (mild, moderate, and severe), which were established on the basis of the PFT results in terms of the mMRC and Borg scale scores (Kruskal–Wallis test, P=0.764). There was no statistically significant difference in the mMRC scores of the groups with and without small-airway obstruction, as determined by the PFT results (median mMRC scores = 0 [0–0], 0 [0–0], respectively; Mann–Whitney *U*-test, P=0.119). There were significant differences in the Borg scale scores of the groups (median scores= 0 [0–1], 0 [0–1], respectively; Mann–Whitney *U*-test, P=0.016).

The results of the univariate analysis indicated that only oseltamivir use and systolic arterial blood pressure (sBP) variables were predictive of the presence of pathology in the 1-month PFT. The logistic regression analysis included oseltamivir and sBP with the following variables, which did not show a significant difference in the univariate analysis, but with a p-value less than 0.2: cough, malaise, presence of diabetes mellitus, and consolidation characterized by CT showed a statistically significant difference. The regression analysis indicated that only the use of oseltamivir detected the presence of pathology in the PFTs in the 1st month (Table 3).

Table 3. Univariate and multivariate analysis of variables for COVID-19 patients' presence of pathology in the 1st month pulmonary function test prediction.

Variables	Abnormal PFT	Normal PFT	Univariate		Multivariate	
	N (%)/ Median (IQR)	N (%)/ Median (IQR)	HR (95%CI)	p-value	HR (95%CI)	p-value
Age, years	47 (40.75–57.5)	51 (45–54)	–	0.735	–	–
Sex (female)	32 (36.8)	55 (63.2)	1.02 (0.58–1.79)	0.950	–	–
BMI>30	26 (34.7)	49 (65.3)	0.88 (0.49–1.58)	0.679	–	–
Symptoms						
Temperature ≥38°C	34 (36.6)	59 (63.4)	1 (0.57–1.75)	0.977	–	–
Cough	37 (42.5)	50 (23.3)	1.52 (0.86–2.66)	**0.147**	1.63 (0.81–3.26)	0.170
Dyspnea	14 (41.2)	20 (58.8)	1.26 (0.6–2.66)	0.544	–	–
Hemoptysis	1 (100)	0 (0)	5.26 (0.21–130.56)	0.366	–	–
Fatigue	40 (41.2)	57 (58.8)	1.44 (0.83–2.51)	**0.199**	1.87 (0.92–3.77)	0.082
Other symptoms	43 (35)	80 (65)	0.85 (0.49–1.49)	0.571	–	–
Comorbid diseases						
Hypertension	15 (37.5)	25 (62.5)	1.03 (0.51–2.1)	0.927	–	–
Diabetes mellitus	14 (51.9)	13 (48.1)	2.02 (0.9–4.56)	**0.085**	1.8 (0.69–4.72)	0.233
Coronary artery disease	4 (36.4)	7 (63.6)	0.98 (0.28–3.45)	1	–	–

Continue...

Table 3. Continuation.

Variables	Abnormal PFT	Normal PFT	Univariate		Multivariate	
	N (%)/ Median (IQR)	N (%)/ Median (IQR)	HR (95%CI)	p-value	HR (95%CI)	p-value
Congestive heart failure	2 (66.7)	1 (33.3)	0.31–39.08)	0.556	–	–
Thyroid disorder	1 (14.3)	6 (85.7)	0.28 (0.03–2.35)	0.265	–	–
Chronic renal failure	3 (60)	2 (40)	2.63 (0.43–16.09)	0.360	–	–
Cigarette smoker (current smoker or quitted)	6 (37.5)	10 (62.5)	1.05 (0.37–2.99)	0.933	–	–
Pack/year ratio in smoking patients	10 (3–20)	14.5 (5.75–20)	–	0.231	–	–
Occupational exposure	3 (42.9)	4 (57.1)	1.32 (0.29–6.03)	0.708	–	–
Medication						
Hydroxychloroquine	78 (37.3)	131 (62.7)	1.19 (0.11–13.35)	1	–	–
Oseltamivir	53 (43.1)	70 (56.9)	1.84 (1.03–3.28)	**0.039**	**2.26 (1.05–4.84)**	**0.037**
Azithromycin	59 (38.6)	94 (61.4)	1.14 (0.76–1.71)	0.529	–	–
Ceftriaxone	22 (37.3)	37 (62.7)	1 (0.68–1.48)	0.996	–	–
Other antibiotics (clarithromycin, meropenem, tazocin, moxifloxacin)	11 (47.8)	12 (52.2)	1.63 (0.68–3.90)	0.271	–	–
Favipiravir	16 (36.4)	28 (63.6)	0.97 (0.49–1.93)	0.926	–	–
Enoxaparine	36 (32.7)	74 (67.3)	0.72 (0.58–1.15)	0.156	–	–
Vital signs						
Heart rate, beats per minute	84.5 (78.8–100.3)	84 (78–92)	–	0.414	–	–
Systolic blood pressure, mmHg	110 (100–120)	110 (110–120)	–	**0.031**	0.98 (0.95–1)	0.090
Diastolic blood pressure, mmHg	70 (65.5–80)	70 (64–80)	–	0.422	–	–
Pulse O_2 saturation, %	97 (94.75–98)	96 (94–98)	–	0.785	–	–
Laboratory results						
Elevated liver enzymes	3 (25)	9 (75)	0.55 (1.15–2.12)	0.540	–	–
Impaired kidney function tests	1 (25)	3 (75)	0.575 (0.06–5.64)	1	–	–
Lymphocyte, $10^3/\mu L$	1.64 (1.27–2.1)	1.45 (1.06–1.94)	–	0.323	–	–
CRP mg/L	1.4 (0.275–5.85)	1.7 (0.3–6.5)	–	0.378	–	–
D-Dimer	522 (348.5–800)	530 (410–890)	–	0.388	–	–
Thorax computed tomography results						
Severe distribution	8 (38.1)	13 (61.9)	1.06 (0.42–2.69)	0.900	–	–
Ground glass opacification	71 (37.4)	119 (62.6)	1.29 (0.47–3.55)	0.618	–	–
Consolidation	37 (32.7)	76 (67.3)	0.68 (0.39–1.2)	0.183	0.80 (0.39–1.64)	0.540
Bilateral distribution	57 (34.8)	107 (65.2)	0.67 (0.34–1.3)	0.233	–	–
Hospital length of stay, days	7 (5–11)	7 (5–12)	–	0.449	–	–
Total time of treatment, days	11.5 (0–19)	11 (6–18)	–	0.563	–	–

Bold values indicate statistically significance.

DISCUSSION

A majority of patients are considered to have recovered from COVID-19. However, residual lung abnormalities have been found 1–3 months after discharge from the hospital[5,6]. Currently, the mechanisms in the long-term effects are considered to be hypoxia or mechanical ventilation-related damage, uncontrolled cytokine release and immune system activation-associated tissue destruction, angiotensin-converting enzyme 2-mediated viral invasion associated direct pneumocyte apoptosis, surfactant inactivation, microvascular or thrombotic disease, and endothelial dysfunction[2,7].

The findings of this study regarding the presence of residual lung abnormalities at follow-up are important. A statistically significant increase in lung dysfunction was observed in the patients who underwent PFTs in both follow-ups (1st and 6th month). The results suggest that all patients with COVID-19 pneumonia should be evaluated for an indication of PFT in the 1-month post-discharge follow-up for residual lung injury. The study also found two issues that influence the need for PFTs. One is a complaint of ongoing dyspnea since the onset of COVID-19 pneumonia. The other is the presence of pathology in the 1-month post-discharge imaging.

Recent studies have reported that restrictive disorders, reduced diffusing capacity, and small-airway obstruction may develop within the first 12 weeks after discharge. Fibrotic changes, including lung fibrosis, have been detected 3 weeks after symptom onset regardless of the severity of the acute illness[2]. Similarly, restrictive disorders and small-airway obstruction were more prevalent in this study. However, some patients also exhibited obstruction of the large airways.

Zhao et al. evaluated recovered patients who were initially admitted with CT abnormalities. They found that 70.91% had radiological abnormalities and 25.45% had lung function abnormalities 3 months after discharge[8]. In this study, 21% of the patients had lung function abnormalities 6 months after COVID-19 pneumonia.

In a recently published study, Guler et al. found lower lung volumes in patients 4 months after severe or critical COVID-19 disease[9]. In this study, no statistically significant respiratory function loss was detected in the 1- and 6-month PFT results of the patients who were considered clinically critically ill during the acute illness period. However, these results might have been influenced by relatively low number of patients in intensive care unit in the study. The statistical analysis indicated a lack of correlation between the severity of the radiologically calculated lung involvement during the acute illness period and the PFT findings. However, FVC and FEV1 were more significantly affected in patients with respiratory distress complaints at the end of the 1st month than in those who did not have these complaints. Again, the negative effects on FVC were evident in those who did not exhibit radiological improvement at the 1-month evaluation.

Another important finding that should be investigated further is the statistically significant lung dysfunction determined in patients whose COVID-19 pneumonia treatment included oseltamivir. The reason might be the use of oseltamivir in combination with other drugs in patients diagnosed as critically ill. However, this could also be a coincidence. Thus, more research is needed.

Limitations

The possibility of underlying asymptomatic chronic lung disease in the patients in the study cannot be completely excluded. In addition, individuals with a body mass index higher than 26.4 have been found to have lower FEV1 and FVC[10]. Due to the small number of obese patients in the current study, subgroup analyses of specific PFT parameters could not be performed.

It is very important that follow-up be done with a higher number of intensive care unit patients. Although our study included a significant number of patients, the number of patients with a history of intensive care hospitalization was relatively low. However, studies have indicated that 80% of patients hospitalized with COVID-19 and 60% of those admitted to intensive care units survive[11]. Perhaps, compared with those who died, the number of patients who were treated in the intensive care unit and discharged from the hospital was relatively low in our study period. Since the number of these patients was not recorded, a clear interpretation could not be made.

The single-center design of the study was also an influential factor in the number of patients with a history of intensive care hospitalization.

CONCLUSIONS

This study indicates the significant role of PFTs in follow-up studies of COVID-19 pneumonia. PFTs can reveal reduced lung function even after the noncritical course of the disease. In summary, patients should participate in follow-ups 1 month after the end of COVID-19 pneumonia treatment. PFTs should be applied to those with sequelae and/or radiological abnormalities of the respiratory tract during these follow-ups. Those with abnormal PFT results should undergo a 6-month follow-up and begin medical management. In addition, the relationship between oseltamivir use and lung damage should be explored in future studies.

AUTHORS' CONTRIBUTIONS

SEE: data curation, formal analysis, investigation, methodology project administration, resources, and supervision, and writing. **SB:** resources, visualization, and roles/writing. **GA:** validation, visualization, and roles/writing. **AE:** resources and writing. **AA:** visualization and writing – review. **MMI:** writing. **SO:** writing.

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ORIGINAL ARTICLE

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Comparison of spermiograms of infertile men before and during the COVID-19 pandemic

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SUMMARY

OBJECTIVE: Since the start of the COVID-19 pandemic, there has been interest in the impact of both SARS-CoV-2 infection and pandemic-induced social restrictions on male reproductive health. This study aimed to evaluate the spermiogram values of men who presented for infertility during the pandemic compared with the previous 2 years.

METHODS: Patients who presented to a urology outpatient clinic for the first time due to infertility were included. The patients' age, semen volume, and spermiogram results were recorded. Based on the presentation date, the patients were divided into prepandemic group 1 (March 2018–February 2019), prepandemic group 2 (March 2019–February 2020), and pandemic group (March 2020–February 2021) for comparison.

RESULTS: A total of 594 patients were included. There was no significant difference between the three groups in terms of the number of patients who presented for infertility (207, 190, and 197 patients, respectively; p=0.691). The mean age was 36.6±7.2 in the prepandemic group 1, 35.5±7.1 in the prepandemic group 2, and 33.1±6.3 in the pandemic group. Patients who presented during the pandemic were significantly younger (p<0.001). There were no differences between the groups in terms of semen volume (p=0.910) or rates of normospermia and pathological spermiogram findings (p=0.222).

CONCLUSIONS: In the first year of the COVID-19 pandemic, there was no significant difference in the number of patients who presented for infertility or in their spermiogram results compared with 2018 and 2019. However, it is noteworthy that the patients were significantly younger during the pandemic than in the previous 2 years.

KEYWORDS: COVID-19 pandemic. Male infertility. Spermiogram. SARS-CoV-2. Semen analysis.

INTRODUCTION

The novel coronavirus disease (named COVID-19 by the World Health Organization [WHO]) has spread from a cluster of unexplained pneumonia cases in Wuhan, China, to a global public health emergency, affecting every country in the world within months, and it was declared a pandemic by the WHO on March 11, 2020[1]. Throughout the intervening year, research has been conducted to understand the health consequences of both SARS-CoV-2 infection and pandemic-induced social restrictions. Before the COVID-19 pandemic, infertility was estimated to affect between 8 and 12% of couples of reproductive age worldwide, with male infertility solely responsible for 20–30% of these cases and a contributing factor in 50% of all cases[2-4]. Since the start of the COVID-19 pandemic, several studies have been conducted on the effect of the SARS-CoV-2 virus on the male reproductive system, especially testicular functions. These studies mostly focused on histopathological changes in testicular and semen parameters caused by COVID-19 infection, the presence of virus in reproductive organs and semen, and its effects on sex hormones[5–8]. However, sexual and reproductive health is not simply an absence of disease or dysfunction but a state of physical, mental, and social well-being related to every facet of reproduction and sexuality[9]. Despite the vaccination

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programs are currently underway, a concern that the pandemic will continue in the long term raises the question of what impact the pandemic-imposed lifestyle changes will have on male reproductive health. Considering the notable changes in sexual behavior observed in society since the start of the COVID-19 pandemic[10,11], community-based studies of male reproductive health during the pandemic are also needed. The results from these studies will be important in further elucidating the social effects of COVID-19.

The aim of this study was to investigate semen parameters in men who presented to the urology clinic due to infertility in the first year of the pandemic and to compare the results with those in the previous 2 years.

METHODS

This study included men who presented to the urology outpatient clinic of the Medical Park Antalya Hospital Complex between March 2018 and February 2021 due to infertility. Inclusion criteria were as follows: the patients who failed to achieve pregnancy for more than 12 months despite regular unprotected sexual intercourse[4] and those who presented to our clinic with this complaint for the first time. Patients who presented for follow-up and those who had previous infertility treatment were excluded from this study.

After obtaining a detailed history and performing thorough physical examination, semen samples were collected in sterile tubes by masturbation after an average of 3–5 days of sexual abstinence and were analyzed after liquefaction at 37°C for 30 min. A 5 µL aliquot of semen was loaded into a Makler counting chamber to determine basic spermatozoa concentration and percentage of motile spermatozoa according to WHO 2010 criteria, and morphological evaluation was performed using light microscopy according to Kruger criteria after staining[12]. Sperm concentration was expressed as sperm per milliliter of semen; motility and morphology were expressed as percentages. The results were evaluated using the following WHO criteria for lower reference limits: semen volume (mL): 1.5 mL; sperm concentration (sperm/mL): 15 million/mL; total motility: 40%; progressive motility: 32%, vitality: 58%, and morphology (normal forms): 4%[12]. All semen samples were evaluated by the same specialist. Patients' age, semen volume, and spermiogram results were recorded. Based on the presentation date, the patients were divided into prepandemic group 1 (March 2018–February 2019), prepandemic group 2 (March 2019–February 2020), and pandemic group (March 2020–February 2021). The results were compared between these groups.

Ethical considerations and statistical analysis

This retrospective study was approved by the Antalya Medical Park Hospital Complex Ethics Committee (approval no: 2021/03) and was carried out in accordance with the Declaration of Helsinki (1975). All statistical analyses were performed using SPSS statistical software (SPSS for Windows version 18.0; SPSS Inc., Chicago, IL, USA). Age and semen volume were expressed as mean±standard deviation. The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Age and semen volume were compared using the Kruskal–Wallis test, and the Mann–Whitney U test with the Bonferroni correction was used for pairwise comparisons. Relative differences in the distribution of normospermia and pathological spermiogram rates by year were compared with the chi-square test. P-values <0.05 were considered statistically significant.

RESULTS

A total of 594 patients were included in the study. The number of patients who presented to the urology clinic for infertility did not differ significantly by year (in chronological order: 207, 190, and 197; p=0.691). The mean age was 36.6±7.2 in the prepandemic group 1, 35.5±7.1 in the prepandemic group 2, and 33.1±6.3 in the pandemic group. Patients who presented during the pandemic were significantly younger (p<0.001). There was no difference in semen volume between the three groups (p=0.910). The analysis of spermiogram results revealed no significant differences in normospermia and pathological spermiogram rates by year (p=0.222). The spermiogram results of all three groups are shown in Table 1.

DISCUSSION

To the best of our knowledge, this study is the first in the literature to evaluate the effects of the COVID-19 pandemic on male fertility by comparing the demographic structure and spermiograms of patients who presented to a urology clinic due to infertility before and during the pandemic. The impact of the pandemic on semen parameters in men is a controversial issue and may occur in two ways. The first one is the effect of the infection itself, while the second one involves the possible psychophysiological effects of pandemic-imposed social restrictions on male reproductive health.

In the pathophysiology of COVID-19 infection, host ACE2 receptors facilitate intracellular entry and replication of the SARS-CoV-2 virus. This process is much easier in cells with high ACE2 expression. In many studies, the ACE2 expression level was found to be high in the seminiferous tubules and in Leydig

and Sertoli cells. This potential affinity of the SARS-CoV-2 virus for the testicles is the main basis for the researchers who support this view. However, studies evaluating the effect of infection on semen parameters have yielded different results. In fact, it remains unclear whether the testicles and other male reproductive organs are susceptible to SARS-CoV-2 infection. Temiz et al.[13] found that sperm quality decreased in the acute period of COVID-19 infection but did not differ from controls after treatment. They attributed the decrease in semen parameters during acute infection to high fever, but noted that SARS-CoV-2 was not detected by polymerase chain reaction (PCR) in the semen during this period[13]. In another study, SARS-CoV-2 was detected by PCR in the respiratory tract but not testicular tissue in postmortem evaluations after COVID-related deaths[14].

The levels of sex hormones in individuals infected with COVID-19 have also been investigated due to their direct effect on semen parameters. Male sex hormones vary dramatically with acute illness or physiological stress, so it is important to remember that these early results may be disputable[15]. In a study of 119 patients, Ma et al. determined that luteinizing hormone and prolactin levels were higher in COVID-19 patients compared with the control group but did not detect a significant difference in testosterone or estradiol levels[7]. In another study, pretreatment levels of follicle-stimulating hormone, luteinizing hormone, and testosterone levels were lower in 30 COVID-19 patients compared with the control group, while posttreatment levels did not differ significantly from those of controls[13]. In a study of 31 patients, Rastrelli et al.[16] reported that testosterone levels were significantly lower in COVID-19 patients who required intensive care than in those with mild disease[16]. It seems that the conversation on the effect of COVID-19 infection on testicular functions cannot be closed yet.

Another important aspect of the COVID-19 pandemic is the major lifestyle changes that have occurred for many individuals. Social isolation during pandemic increased the feeling of loneliness and the usage of mobile devices, laptops, and computers. These devices may adversely affect semen parameters due to the low-level exposure of radio-frequency electromagnetic fields they produce[17]. In addition, obesity that can occur due to a sedentary lifestyle may also be an important risk factor. A meta-analysis study showed that semen quality was lower in obese men than in men with normal weight[18]. Psychosocial stress caused by the pandemic also constitutes a significant public health problem. Actually, the effect of psychosocial stress on semen parameters has been a topic of debate for a long time. In a prospective study, Hjollund et al.[19] determined that there was no relationship between stress and sperm parameters[19], whereas Janevic et al.[20] found a negative association between stress and sperm concentration, motility, and morphology[20]. As the duration and degree of stress experienced by a lonely man cannot be measured with objective parameters, the effect of stress on semen parameters remains uncertain. The results of the present study suggest that these pandemic-related risk factors are not severe enough to disrupt sperm parameters. Of course, these 1-year results can also be seen as a preliminary study. If the pandemic continues, future studies of larger series will provide more insight into this issue.

It is also necessary to question the sex life of patients who present due to male infertility during the COVID-19 pandemic. Analyzing the semen parameters alone may not be enough in these patients. It has been observed in pandemics that patients affected by mental disorders far outnumber the infected patients[21]. Therefore, it is important to remember that increased anxiety or depression in men during the pandemic can also cause erectile dysfunction (ED) and loss of libido[22]. An individual's psychological state may trigger or exacerbate ED. Depression has been found to double the risk of ED[23]. ED may in turn cause a loss of self-esteem, which can adversely affect

Table 1. Spermiogram results by year.

	Prepandemic group 1 (2018)	Prepandemic group 2 (2019)	Pandemic group (2020)	p-value
Number of patients	207	190	197	0.691
Age (years), mean±SD	36.61±7.21	35.58±7.15	33.15±6.36	<0.001
Semen volume (mL), mean±SD	3.96±1.74	4.09±1.93	4.09±1.78	0.910
Normospermia rate (%, n)	29 (60)	27.4 (52)	35 (69)	
Oligoasthenoteratoozospermia (%, n)	24.2 (50)	25.8 (49)	17.3 (34)	
Asthenoteratoozospermia (%, n)	16.9 (35)	17.4 (33)	18.8 (37)	
Asthenoozospermia (%, n)	11.6 (24)	13.7 (26)	16.8 (33)	
Teratozoospermia (%, n)	10.1 (21)	7.4 (14)	6.6 (13)	
Cryptozoospermia (%, n)	2.4 (5)	3.2 (6)	1.5 (3)	
Azoospermia (%, n)	5.8 (12)	5.3 (10)	4.1 (8)	

their partner relationship. Therefore, performing a psychological evaluation is recommended before seeking an organic cause for ED, especially in men under the age of 40[24].

Another important issue during the pandemic is loss of libido[9]. Although this condition (also known as hypoactive sexual desire disorder) is less common in men than women, it can lead to important problems between couples[25]. In a prevalence study by Carvalheira et al.[26], loss of libido lasting more than 2 months was most commonly seen among men aged 30–39 years[26]. Clinicians should bear in mind that low libido is not only caused by low testosterone levels, but can also occur as a side effect of antidepressant and antipsychotic drugs[27]. Furthermore, sexual performance anxiety, relationship problems between couples, and depression/anxiety reduce sexual desire[28].

One of the important findings of our study was that the mean age of men presenting due to infertility during the pandemic was 33.1±6.3 years, significantly younger than in the previous 2 years. This difference may reflect an increase in future anxiety in these patients during the first year of the pandemic. A study in the literature demonstrated that the prevalence of anxiety/depression during the COVID-19 pandemic increased significantly more among participants <35 years of age than in participants >35 years of age[15]. According to the definition of infertility, pregnancy is not achieved for 12 months despite timed or regular sexual intercourse. Therefore, a detailed history should be obtained from infertile couples to understand the frequency of sexual intercourse during the COVID-19 pandemic. The lack of difference in the number of patients presenting to the clinic due to infertility compared with the previous 2 years indicates that there was no change in couples' desire to have children, despite the adverse circumstances brought about by the COVID-19 pandemic.

CONCLUSION

The available data suggest that for patients who present due to infertility during the pandemic and have no major problems with semen parameters, a thorough history should be obtained, and psychiatric evaluation is performed to question about sexual desire and presence of ED.

The limitation of this study is the lack of data regarding whether the patients who presented during the pandemic had a history of COVID-19 infection. However, the main objective of this study was not to evaluate the effect of COVID-19 infection specifically but to demonstrate the overall impact of the pandemic. Therefore, this study did not examine the history of COVID-19 infection in the patients who presented due to infertility during the pandemic. Moreover, the results of this study pertain to the first year of the pandemic. As the pandemic continues, the results of future long-term studies will be more valuable. In addition, specific sperm changes were not evaluated and dynamic analysis was not performed in this study[2,3].

During the first year of the COVID-19 pandemic, there was no significant difference in the number of men who presented to the urology outpatient clinic due to infertility or in the patients' pathological results in terms of semen volume and sperm count, motility, and morphology when compared with data from 2018 and 2019. However, the men who presented for treatment during the pandemic were significantly younger than those in the previous 2 years. Questioning about psychosexual behavior may also be considered when evaluating infertile men during the pandemic.

ETHICAL APPROVAL

The study protocol was approved by the local ethics committees.

AUTHORS' CONTRIBUTIONS

MS: Conceptualization, Methodology, Writing – original draft. **MD:** Data curation, Resources, Writing – review & editing. **ME:** Formal Analysis, Software. **EYK:** Funding acquisition. **SSU:** Investigation, Validation, Visualization. **AS:** Project administration. **HT:** Supervision.

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ORIGINAL ARTICLE

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Constipation in the period of limited isolation during COVID-19 pandemic

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SUMMARY

OBJECTIVE: COVID-19 outbreak has become widespread globally and caused a new global chaos. This outbreak that completely affected the lifestyle of individuals resulted in periods of isolation. Here, we evaluated the effects of lifestyle changes with isolation on constipation.

METHODS: A survey on constipation was performed during the 12-week isolation period starting in March 2020 in Turkey. Data of 390 individuals who participated in the survey through the social media and who were actively employed prior to isolation were analyzed. Rome IV criteria were used to evaluate constipation.

RESULTS: Among the participants in the study, 253 (64.9%) were women with the mean age of 39.5±9.5 years. A statistically significant association was found between the decreased water consumption during the isolation period and constipation after the isolation ($p=0.020$; $p<0.05$). A significant association was found between the changes in physical activity and constipation after the isolation ($p=0.013$; $p<0.05$). New development of constipation during the isolation or declaration of increased constipation was found to be statistically associated with post-isolation constipation according to Rome criteria ($p=0.000$; $p<0.05$).

CONCLUSION: The data of this present study demonstrated that isolation period was effective on the newly developed constipation. Decreased physical activity and water consumption are also effective on constipation.

KEYWORDS: Constipation. Pandemic. COVID.

INTRODUCTION

A new coronavirus (COVID-19) was reported to infect humans through droplet spread starting from China in December 2019 rapidly to other countries and caused deaths due to respiratory failure. Therefore, World Health Organization (WHO) declared the situation as a pandemic on March 11, 2020[1]. In addition, many countries implemented various preventive measures and limitations such as social distancing rules and intermittent quarantine in order to prevent or slow down the spread of the virus. Quarantine has been reported to have negative effects such as psychological stress, insomnia, low mode, and post-traumatic stress disorders on individuals as was known in the SARS era[2]. In addition, it was demonstrated that the risk of eating disorders is increased during quarantine, in contrast to adaptation to healthier nutrition[3,4].

Constipation is a disorder of the gastrointestinal system and is defined as decreased frequency of stools, hard defecation, excess strain, decreased stool passage, or sensation of incomplete evacuation and could be developed due to isolation or underlying disorders[5]. Rome IV diagnostic criteria have been used to evaluate the changes in defecation and Bristol stool scale for the categorization of the shape of the stools[6].

Incidence of constipation in this country is around 20%, according to a study performed[7]. Median prevalence in the world is 16% and constipation is frequently seen in female gender aged above 60 years. Constipation is associated with sedentary lifestyle, nutrition composed of low fibers, drugs, anxiety, depression, and somatization as well as changes in the lifestyle[5,6,8,9]. Also, quality of life of individuals is negatively associated with constipation[10]. Thus, since limiting people to home causes negative changes in eating habits, psychological conditions, and physical activity level, these negative effects might have negative impact on defecation habits as well.

The aim of this cross-sectional and online survey study was to evaluate whether the rate of constipation was changed in this country among the employed individuals during the COVID-19-associated isolation period. The first case of COVID-19 in Turkey was declared on March 11, 2020, the day when WHO first declared the pandemic. Schools were closed on March 16, 2020 and education was turned to online style gradually[11]. Cafes and restaurants were closed on March 21, 2020, services were changed to takeaway[11], and intense precautions were implemented using an application called "stay home." A partial quarantine was declared from March 2020 to May 2020. Many firms started to implement home office working

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order and flexible working hours were implemented for public employees[12]. The total number of cases in this period was 141,475 with 3,894 deaths. As a result, majority of the population stayed home for 12 weeks[11].

METHODS

This cross-sectional survey study was designed as online questions and was used to evaluate the changes in the rate of constipation among the employed individuals during the limited quarantine period in the COVID-19 outbreak. It was performed in Turkey, in which Istanbul was the city with the highest number of cases. Although 404 individuals answered the survey questions, the results of 390 individuals were included in the evaluation after exclusion of the remaining responses due to duplicate answers and mismatch with the inclusion criteria. Ethics board approval was obtained from local ethics board committee (number: HNEAH-KAEK 2020/KK/109). Also, the study was conducted in accordance with the ethical standards of Declaration of Helsinki (revised in 2013). The study was conducted through the social media (e.g., WhatsApp and Twitter) preparing Google forms between June and July 2020. Forms filled in by the individuals were transferred to the authors by email. A group of individuals who were 18 years or older, had no chronic drug use interfering with the gastrointestinal (GI) system, and actively employed prior to the quarantine were included in this study. Individuals who were using drugs that cause constipation or who were under the treatment of constipation, who were not actively employed, had immobilization for any reason (e.g., cerebrovascular disease [CVD] and major depression), had GI surgery, and who refused to participate in the study were excluded from the study. A total of 36 questions were present in the survey. The questions were designed by the authors including questions on the demographic data of the group included, groups of occupation, accommodation during the isolation period, changes in the eating habits, physical activity status, sleep and stress conditions, presence of symptoms of constipation before (using Rome IV criteria)[13] and its duration, if present, changes in defecation habits, stool shapes (using Bristol scale)[10], and drug use in the newly developed constipation. In contrast, chronically used drugs, prior GI surgery, and comorbidities of the individuals were questioned.

Statistical analysis

IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used in the statistical analyses when evaluating the findings of the study. Descriptive statistical methods (mean, standard deviation, and frequency), in addition to chi-square test, Fisher's exact chi-square test, continuity (Yates) correction, and McNemar test, were used in the comparison of qualitative data. A p-value <0.05 was considered statistically significant.

RESULTS

The study was conducted in a total of 390 individuals with an age between 17 and 67 years and with 253 (64.9%) females and 137 (35.1%) males. Mean age was 39.5±9.5 years. A total of 66 (16.9%) participants had a chronic disease. A majority of the employed participants were teachers (n=118, 30.25%).

According to the data shown in Table 1, the rate of straining in at least one-fourth of the defecations was 29.7% prior to the isolation, while this rate was found to statistically significantly regress to 22.6% after the isolation (p=0.001; p<0.05). The rate of frequency of defecation less than three times a week was 5.9% prior to the isolation, while this rate was found to statistically significantly regress to 2.8% after the isolation (p=0.004; p<0.05). Rate of rare soft stools without laxative use was 8.5% prior to the isolation, while this rate was found to statistically significantly regress to 5.6% after the isolation (p=0.019; p<0.05). Number of cases with constipation according to Rome criteria prior to the isolation was 59 (15.1%), while it was 48 (12.3%) after the isolation. No statistically significant difference was observed in the general rate of constipation between the periods before and after isolation (p>0.05).

The answer to the question "was there any newly formed constipation or any increase in the pre-existing constipation?" was "yes" in 15.4%. Among 60 individuals who responded "yes," 18.3% stated that they started to use drugs for this reason or they increased the frequency of drug use.

Questions related with constipation after the isolation are presented in Table 2. A statistically significant association was found between the decreased water consumption during the isolation period and constipation after the isolation period (p=0.020; p<0.05). A statistically significant association was found between the changed physical activity during the isolation period and constipation after the isolation period (p=0.013; p<0.05). The rate of constipation in individuals with a decreased physical activity (15.1%) was significantly higher compared with the ones with increased activity (10.2%) and unchanged activity (1.6%).

New development of constipation during the isolation or declaration of increased constipation were found to be statistically associated with post-isolation constipation according to Rome criteria (p=0.000; p<0.05). Among the individuals who

Table 1. Evaluation of the change in defecation symptoms after isolation compared with before isolation.

		Yes		No		p
		n	%	n	%	
Straining during more than ¼ (25%) of defecations	Before isolation	116	29.7	274	70.3	0.001*
	Post isolation	88	22.6	302	77.4	
Lumpy or hard stools more than ¼ (25%) of defecations	Before isolation	62	15.9	328	84.1	0.699
	Post isolation	58	14.9	332	85.1	
Sensation of incomplete evacuation more than ¼ (25%) of defecations	Before isolation	43	11	347	89	0.883
	Post isolation	45	11.5	345	88.5	
Sensation of anorectal obstruction/blockage more than ¼ (25%) of defecations	Before isolation	32	8.2	358	91.8	0.061
	Post isolation	21	5.4	369	94.6	
Manual maneuvers to facilitate more than ¼ (25%) of defecations	Before isolation	24	6.2	366	93.8	0.227
	Post isolation	19	4.9	371	95.1	
Fewer than three spontaneous bowel movements per week	Before isolation	23	5.9	367	94.1	0.004*
	Post isolation	11	2.8	379	97.2	
Loose stools are rarely present without the use of laxatives	Before isolation	33	8.5	357	91.5	0.019*
	Post isolation	22	5.6	368	94.4	

McNemar test. *p<0.05.

stated that they had new development of constipation during the isolation or had an increased rate of constipation, 40% had constipation after the isolation. Among the individuals who declared development of no new constipation, 7.3% had constipation after the isolation (Table 3).

DISCUSSION

Functional constipation has been demonstrated to negatively affect the quality of life in many studies[14,15]. The term constipation is perceived differently among the patients and, thus, many individuals who define themselves constipated might actually not accepted as constipated according to the Rome criteria[13-16].

In a survey including 5,155 individuals, only half of the participants thought that constipation was a health-related problem[17]. Physical activity, improving fluid intake when decreased, and fiber-rich nutrition are known to be effective in preventing constipation[14,18]. No relationship could be established between fiber-rich nutrition and constipation. Yet, fiber-rich nutrition is recommended in the treatment of functional constipation[19]. Among the participants in the survey, 74.1% reported a decreased rate of take-home food, although a partial increase (33.8%) in the consumption of preprepared food intake was the subject. However, fiber-rich food intake was unchanged clearly in 262 (67.2%) individuals. This suggests that individuals increased cooking home due to the isolation and also fear of catching the disease and they balanced their nutrition in favor of fiber-rich food.

The significant association of Rome criteria confirmed rate of newly developed constipation and isolation demonstrates that isolation period was effective on constipation. Decreased physical activity and water consumption are also effective on constipation. Constipation, especially when it becomes a chronic problem, causes nonignorable costs in the healthcare system and labor loss[15]. Considering possible future isolation due to pandemic, evaluation of some organ system dysfunctions such as constipation during the isolation period and preventive measures against them will allow labor loss of the personnel with a decreased work tempo and the economical costs might be eased. Although many drugs can be prescribed for constipation, many patients take them in a decreased dose or frequency due to side effects or stop using the drugs and many physicians prescribe drugs more than required[16]. The first approach for the treatment of constipation should be changing eating habits and lifestyle[20]. Increasing water intake for constipation that would possibly develop during the isolation period is beneficial for an individual in many ways and is an easy-to-apply method of initial treatment.

Increased stress causes differences in gastrointestinal system habits[21]. Constipation has been associated with stress in some studies; however, the rate of constipation was not significantly increased in the group of individuals who stated an increased stress with isolation in this present study[18,22].

Table 2. Evaluations of post-isolation constipation.

		Post isolation constipation according to Rome 4 Criteria		p
		Yes	No	
		n (%)	n (%)	
Accommodation elsewhere during the isolation period	Yes	6 (9.4)	58 (90.6)	0.567[a]
	No	42 (12.9)	284 (87.1)	
Accommodation (n=64)	House	5 (14.3)	30 (85.7)	0.209[b]
	Others	1 (3.4)	28 (96.6)	
Number of meals during the isolation period	Increased	19 (15.3)	105 (84.7)	0.455[c]
	Decreased	7 (11.7)	53 (88.3)	
	Unchanged	22 (10.7)	184 (89.3)	
The amount of food ordered from outside during the isolation period	Increased	1 (5.9)	16 (94.1)	0.695[c]
	Decreased	37 (12.8)	252 (87.2)	
	Unchanged	10 (11.9)	74 (88.1)	
Food consumption during the isolation period	Increased	24 (13)	160 (87)	0.901[c]
	Decreased	5 (10.9)	41 (89.1)	
	Unchanged	19 (11.9)	141 (88.1)	
Packaged product consumption during the isolation period	Increased	19 (14.4)	113 (85.6)	0.477[c]
	Decreased	10 (9.3)	98 (90.7)	
	Unchanged	19 (12.7)	131 (87.3)	
Water consumption during the isolation period	Increased	28 (13.8)	175 (86.2)	0.020[c]*
	Decreased	6 (28.6)	15 (71.4)	
	Unchanged	14 (8.4)	152 (91.6)	
Physical activity status during the isolation period	Increased	5 (10.2)	44 (89.8)	0.013[c]*
	Decreased	42 (15.1)	237 (84.9)	
	Unchanged	1 (1.6)	61 (98.4)	
Consumption of fibrous food during the isolation period	Increased	12 (13.2)	79 (86.8)	0.159[c]
	Decreased	8 (21.6)	29 (78.4)	
	Unchanged	28 (10.7)	234 (89.3)	
Daily sleep time during the isolation period	Increased	26 (14.8)	150 (85.2)	0.221[c]
	Decreased	9 (14.1)	55 (85.9)	
	Unchanged	13 (8.7)	137 (91.3)	
Stress situation felt during the isolation period	Increased	37 (13.3)	242 (86.7)	0.290[c]
	Decreased	1 (3.3)	29 (96.7)	
	Unchanged	10 (12.3)	71 (87.7)	

[a]Continuity (Yates) fix. [b]Fisher's exact test. [c]Ki-kare test. *p<0.05.

Table 3. Evaluation of the relationship between the increase in new constipation caused by isolation and post-isolation constipation according to the Rome criteria.

		Constipation after isolation according to the Rome criteria		p
		Yes	No	
		n (%)	n (%)	
New constipation or increased constipation associated with isolation	Yes	24 (40)	365 (60)	0.000*
	No	24 (7.3)	306 (92.7)	

Ki-kare test. *p<0.05.

The effects of acute and chronic stress on intestinal function may vary. Stress causes an increase in the contraction activity of the intestines. Chronic stress has been associated with inflammatory bowel disease and irritable bowel disease. Stress might result in constipation or diarrhea. The isolation period evaluated was a process of 3 months, and analysis of the effect of chronic stress in isolation in longer periods might have different results[23]. Evaluation of the association of constipation and chronic stress might be beneficial since constipation is known to have a psychological component[14].

Many constipated patients who deny to present to the health care facilities and who refuse to share their personal information might participate in the surveys safely. Especially, the employed fraction of the society reach Internet easily and use it actively. Thus, the group reached through the Internet in order to obtain data on the problem of constipation has the qualification to reflect the employed fraction of the society[17].

This study may not reflect the whole society since only the group of employed people were included in the survey. Low socioeconomic status and low level of education are risk factors for constipation[24]. The nutritional habits and thus rates of constipation may not reflect the whole society since the participants in the survey had a definite level of income and level of education. However, we suggest that the group of participants in the survey reflected the employed part of the society as well, since the aim of this study was to evaluate the rate of constipation in the employed fraction of the society with isolation.

CONCLUSIONS

The rate of newly developed constipation is increasing in the isolation period. Increasing physical activity and water intake might solve the problem as a first step in order to improve the quality of life and increase work productivity in the future possible isolation condition.

AUTHORS' CONTRIBUTIONS

SAK, OZS: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision.

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ORIGINAL ARTICLE

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Ethical and bioethical aspects concerning the disclosure of medical information for a fair reason

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SUMMARY

OBJECTIVE: The objective of this study was to emphasize the importance of legal and bioethical knowledge in maintaining medical confidentiality, especially in situations when there is a diagnosis of HIV infection.

METHODS: A literature review of studies published in the Scientific Electronic Library Online and National Library of Medicine databases was performed. Sixteen studies available in full, online, and free, published between 2010 and 2020, were selected.

RESULTS: The studies highlighted that, despite the ethical duty to breach confidentiality for the protection of third parties, many doctors are reluctant to reveal this secret due to the power of stigmatization and social discrimination related to the diagnosis of HIV infection, which affects integrity, counseling, and capability to treat patients.

CONCLUSION: HIV diagnosis implies bioethical and legal questions. Respect for medical confidentiality is a matter to be discussed, as there is a need to protect the privacy of the patient, at the same time the responsibility to preserve the health of others.

KEYWORDS: Confidentiality. Bioethics. HIV. Human rights.

INTRODUCTION

According to article 73 of the Brazilian Code of Medical Ethics, physicians are prohibited from disclosing information obtained in the exercise of their professional activities and must maintain confidentiality in any situation, except for a fair reason, a legal duty, or written consent from the patient. If this ethical command is violated, the professional is subject to administrative sanctions applied by the Brazilian Federal Council of Medicine. Also, the Brazilian Penal Code, in its article 154, provides for the preservation of professional secrecy, criminalizing the conduct of breaking the secret[1,2].

However, the same professional code provides exceptions to this imperative of secrecy. Situations in which there is express authorization from the patient, the law requires the disclosure of information (such as in cases involving suspicion of mistreatment of children and adolescents in which the Child and Adolescent Statute determines the communication of information to the Council Guardianship), or when there is just reason, confidentiality can (and should) be breached. Accordingly, in cases where the patient has an infectious disease, in which there is a risk of contamination from third parties, there is a need for communication from third parties, at the risk of contagion, morbidity, and mortality of these people[3]. In this sense, the Brazilian Penal Code itself, in its article 130, provides for a penalty of 3 months to 1 year of detention and a fine for those who "expose someone, through sexual intercourse or any libidinous act, to contagion of venereal disease, of who knows or should know that he is contaminated."

The Brazilian Federal Council of Medicine, aware of these issues, published Resolution 1665/2003 which provides that professional secrecy must be strictly respected in relation to patients with the HIV virus, but still noting that this secrecy can be broken for a fair reason[3,4].

In contrast, the American Medical Association advises physicians to comply with disease notifications but preserve the confidentiality of the patient's condition. Thus, when a patient's illness represents a threat to another identifiable individual, the physician's duty is to notify public health authorities and communicate the risk to third parties, without, however, revealing the identity of the infected person. The justification for such a recommendation is to guarantee protection to HIV-infected patients since there is still great social discrimination[3,5].

This study aims to identify in the literature the different approaches related to medical confidentiality and discussions

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about exceptions to disclosure of information for a fair reason, especially when there is a risk to the health of third parties.

METHODS

This study was carried out from a query made on the Scientific Electronic Library Online and US National Library of Medicine data platforms using the Health Sciences Descriptors. The key words used were "HIV," "AIDS," crossing them with the terms "confidentiality," "bioethics," "medical confidentiality," and "medical ethics," in Portuguese, English, and Spanish. Boolean operators were also used to increase the quality of the results: "HIV and medical ethics," "Breach of medical confidentiality," "HIV and medical confidentiality," and "HIV and confidentiality."

Studies that were published between 2011 and 2020 were included. Duplicate articles, that were published outside the stipulated period, and that did not meet the research objectives were excluded.

RESULTS

Sixteen studies were selected which constituted two main research focuses: (i) ethical and bioethical dilemmas in the management of patients with HIV infection and (ii) violation of medical confidentiality for a fair reason.

In the analysis of the selected articles, approaching the panorama of ethical and bioethical dilemmas in the management of HIV-positive patients, the power of stigmatization and social discrimination in the care of these patients can be highlighted, which directly affects their integrity, the stimulus for realization testing, counseling, and treatment of the disease. From this perspective, Mataboge and collaborators pointed out the inefficiency of the public health system in South Africa, which creates tensions between individuals with acquired immunodeficiency syndrome and physicians, whether due to the lack of clinical and laboratory approach to the disease, or the conditions of continuity of treatments, due to inadequate ethical and moral training of these health professionals, added to society's lack of information about the dilemmas related to HIV infection, which directly affects the impact of the infection in the country[6]. In line with these findings, Dapaah and Senah pointed out that stigma is the main underlying reason that explains the reluctance of many people to undertake voluntary testing and accept medical advice, which would lead to adequate and early antiretroviral treatment. For this population, infection would be associated with death and not with sexual promiscuity, so that stigmatized patients believe that the disease is incurable and that their pain and suffering are shameful[7].

Arrivillaga-Quintero highlighted tensions and barriers related to adherence to antiretroviral treatments by infected women in Colombia, showing that the problems faced by women are even greater than those faced by men; according to the author, the low adherence verified in the country is structurally determined by the health system, either by the lack of rights and comprehensive health care, or by the violation of confidentiality of information or by social discrimination. It was evident that women affected by HIV infection are stigmatized, being objects of rejection because of this diagnosis and face more discrimination than men; that the possible breach of confidentiality by professionals generates even greater tensions, making adherence to treatment difficult[8].

Domínguez and collaborators discussed the application of the bioethical principles of autonomy, beneficence, non-maleficence, justice, in addition to the challenges of diagnosing HIV infection to these principles. According to them, in these circumstances, the physician is faced with the dilemma of choosing between the individualistic ethics typical of his deontology and a series of social needs that demand other types of moral behavior, even though in practice, the obligation, both moral and legal, to inform the spouse/partner of the patient's situation, and communication to the health authorities is also mandatory. From the authors' perspective, violations of medical confidentiality can discourage patients from testing and adhering to treatments due to fear of discrimination and exposure to others. So, it is the responsibility of health professionals to provide comprehensive care to these patients and strictly observe the ethics and dilemmas that arise in their daily lives[9].

In a study by Silva and Ayres, these authors confirmed that, in case the patient refuses to inform his/her partner about the diagnosis, it is the duty of health professionals to disclose the information. In situations of conflict, the physician must be guided by ethical and legal principles because, despite the importance of preserving confidentiality with the patient, in this case, it is necessary to protect the sexual partner from contamination by the virus[10].

According to the Opinion of the Regional Council of Medicine of Rio de Janeiro number 16/92, prepared with the assistance of the Technical Advisory Committee for the Prevention, Control, and Treatment of AIDS, the intensity of the harm caused to the patient by the breach of confidentiality must be evaluated. Thus, if the harm caused to the patient by breaking this confidentiality is greater than that potentially caused to the partner if he were to be infected, the medical confidentiality should be maintained[11]. Villas-Bôas highlighted key points of the duty of professional secrecy in health, supporting the necessary trust in the doctor–patient relationship. In this

issue, the exceptions of breach of confidentiality for a fair reason in cases of HIV diagnosis are addressed. The disclosure is expected to take place in a restricted scope, so that the epidemiological care and conduct do not represent unnecessary exposure and source discrimination and embarrassment to the patient.

From another point of view, Burger dealt with issues related to the lack of registration of HIV infection as the underlying cause of death on the death certificate, justifying the issues of patient confidentiality and undue exposure of the diagnosis, which could impact the issues of life insurance, funeral policy claims, beliefs, etc. Therefore, the author stated that, in these administrative matters, a decision must be taken which balances the consequences of not breaking medical confidentiality and the consequences of disclosing the diagnosis to the sexual partners of the deceased[12].

DISCUSSION

Specifically, when it comes to the diagnosis of HIV infection, several studies address the difficulties of maintaining medical confidentiality when stigma, prejudice, and social discrimination have taken root in society. Other authors emphasized that the main cause of stigma would be the fact that the disease is associated with death and not sexual promiscuity. In view of this, a study carried out in Ghana argues that HIV-positive patients are discriminated because of the belief that the disease is incurable and that their fears and feelings are said to be shameful[6]. According to a study by Arrivillaga-Quintero, it is the influence of social class on this theme; according to the author, women in higher social positions experience greater stigma when compared with women in less privileged social positions. This fact would increase the fear of the breach of confidentiality by women who are socially better placed, which would make them opt for private services, to the detriment of public services, in order not to be enrolled in the system. This would reveal the patients' lack of trust in the health professionals responsible for providing care in public health services[8].

From another angle, Bernal and Álvarez traced the trajectory of the ethical dilemmas of confidentiality and the handling of the HIV diagnosis in the face of notifications of positive results identified to the health departments and the controversies related to the exposure of sexual partners. Again, the authors highlighted the difficulties of reconciling professional secrecy by physicians and the health care team with the ethical requirement to avoid harm to those people who, kept in strict professional secrecy, can become infected, and are in danger of dying. However, they also reinforce the guidelines of the American Medical Association that professional secrecy admits exceptions as long as the doctor prevents the spread of contagious diseases but recognizes the right to confidentiality of people with HIV. According to the authors, the physician should try to persuade the infected patient to report the case to the sexual partner, and if unsuccessful, the authorities should be informed; if further steps are not taken, the physician must finally inform and advise that third person is at risk[13].

According to the Brazilian Ministry of Health (2013), the AIDS acts destructively on the immune system, having high severity and great relation to the appearance of opportunistic infections and neoplasms. Therefore, the ethical and bioethical discussion of the flexibility of professional confidentiality is essential, when there is a possible risk to the sexual partner's life, to avoid new transmissions, and to promote awareness and treatment of the disease. The questions about the maintenance of medical confidentiality increase when there is a risk to the health of other people. Again, the feasibility of placing HIV infection as the primary cause on the death certificate is discussed and the fact should be disclosed to the funeral policy insurer, the life insurance company, and the sexual partner. In this bias, the Federal Council of Medicine recommends that, even after death, medical secrecy must be respected, again except in cases of compulsory notification, situations that involve risk to others, and mistreatment of minors. Therefore, according to the Federal Council of Medicine of Brazil, if the patient's diagnosis represents a threat to another identifiable individual, the doctor's duty ranges from notifying public health authorities to communicating the risk to third parties, without revealing the person's identity source. Thus, the breach of confidentiality is justified, given that medical secrecy, undeniably, serves to protect the HIV-infected patient from social discrimination, with the proviso that it should not contribute to the spread of the virus.

Finally, Claudia Mora presented a study suggesting that counseling, pretesting, and obtaining specific informed consent for the exam represent barriers to acceptance of the test, as they increase anxiety in patients. Thus, counseling would only be recommended at the time of delivery of the result, with the aim of reducing the stigma in relation to testing, and minimizing the constraints arising from the spontaneous search for health services[14].

CONCLUSIONS

The importance of knowledge of ethical, bioethical, and legal norms related to the maintenance of professional secrecy is evident, especially considering the impact of disclosure of confidentiality in cases of HIV infection, as there

are implications for social discrimination in stigmatized patients, which can discourage patients and directly affect the demand for health services, anti-HIV testing, treatment, and medical reliability, given all the feelings of doubts and fears rooted in society.

AUTHORS' CONTRIBUTIONS

GCR, LLG: Conceptualization, Data curation, Visualization, Writing – original draft. **FRC:** Formal Analysis, Investigation, Supervision, Validation. **FRC, GCR, LLG:** Methodology, Project administration. **ANCM, FRC, MO:** Writing – review & editing.

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ORIGINAL ARTICLE

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Prevalence of COVID-19 in medical school and residency in Porto Alegre, RS

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SUMMARY

OBJECTIVE: A multicentric, cross-sectional study was carried out to determine the prevalence and risk factors for Coronavirus disease 2019 in medical students and residents from four universities and affiliated hospitals in Brazil.

METHODS: A survey about contamination risk and symptoms was sent to all participants through email and WhatsApp. Prevalence was measured by the self-report of positive polymerase chain reaction or serological test. Univariate and multivariate analyses were performed, and odds ratio and 95% confidence interval were calculated.

RESULTS: Prevalence of infection by Sars-CoV-2 was 14.9% (151/1011). The disease was more prevalent in residents and interns than in undergraduate students. Contact with an infected relative outside the hospital or with colleagues without using personal protective equipment was associated with higher contamination. Contact with patients without wearing goggles and higher weekly frequency of contact were the two factors independently associated with the infection by Coronavirus disease 2019 in the multivariate analysis.

CONCLUSIONS: Medical students, interns, and residents have a higher prevalence of Coronavirus disease 2019 than the general population, in which the last two groups are significantly at higher risk. Contacting patients at a higher weekly frequency increases the risk for infection. The use of goggles should be reinforced when contacting patients.

KEYWORDS: Sars-CoV-2. Prevalence. Students, Medical. Medical Residency. Internship.

INTRODUCTION

The pandemic of the coronavirus disease 2019 (COVID-19) caused by Sars-CoV-2 emerged in China and can clinically vary from a mild cold to a multisystemic disease[1]. The disease is manifested by a variety of symptoms such as fever, dry cough, dyspnea, fatigue, body aches, headaches, anosmia, and ageusia which usually progress to spontaneous remission[2]. According to COVID-19 data from the World Health Organization (WHO), from September 07, 2021, over 221 million cases were confirmed and more than 4 million deaths occurred worldwide, and 12.4 million confirmed cases and over 500,000 of those deaths occurred in Brazil[3]. New hygiene habits were adopted from public policies recommended by WHO, including the use of masks and social distancing[4]. As part of these measures, on-site classes were also suspended and the traditional learning strategies underwent several changes at all levels of education in Brazil including medical courses[5,6].

Healthcare professionals face a greater risk of infection. A study carried out in a hospital in Wuhan identified an infection rate of 29% among healthcare professionals[7]. Investigations about deaths of doctors related to the infection due to their work on the front line showed fatalities of professionals from all healthcare areas, especially in men with an average age of 63.5 years[8]. A study from the time of the previous SARS pandemic showed that medical students who did not have

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contact with patients did not contract SARS[9]. The purpose of this study was to determine the prevalence of COVID-19 among medical students in different stages of their medical education. The prevalence of COVID-19 in this group of people was evaluated in regard to the use of personal protective equipment (PPE), as well as to the frequency of exposure and to the point-of-care of patients in general and of patients knowingly contaminated.

METHODS

This is a multicentric, cross-sectional study carried out in four medical schools located in the Porto Alegre (Brazil) and their respective teaching hospitals from August to December 2020. Both positive polymerase chain reaction (PCR) and serological tests were considered COVID-19 infection. The prevalence of the virus was compared in three groups: undergraduate students (1st to end of 4th year of study), interns (5th and 6th year of study), and residents from four Medical Schools. Additionally, the prevalence of COVID-19 was evaluated according to the use of PPE (isolation gowns, N95 masks, caps, goggles, and face shields), the frequency of exposure (months of the year and weeks in the last month), and the point-of-care (outpatient facility, emergency care settings, intensive care unit [ICU]) of patients in general as well as of patients known to be infected (a positive PCR test). The selection of the sample included all the undergraduate students and interns enrolled in these universities and the residents from the respective affiliated hospitals contacted through email and WhatsApp. All students, interns, and residents who completed the questionnaire within the allotted time for the data collection were included in the study. Data were tabulated and double-checked. Variables were analyzed in a descriptive way. Descriptive analysis was performed for quantitative variables (averages, standard deviation, and medians). For the qualitative variables, absolute and relative frequencies were calculated. The chi-squared test and the Fisher's exact test were applied to test homogeneity among the proportions. The univariate and multivariate logistic regression model was employed to assess COVID-19 predictors and the odds ratio, using SPSS, version 17.0 (Chicago, IL, USA). The significance level used for the tests was 5%. All participants signed an informed consent, and data were kept confidential. The research was approved by the ethics committee/institutional review board of the coordinating center and the other participant universities (CAAE number 36498920.3.1001.5345). The individual projects were forwarded with the endorsement of the clinical management from all four participant hospitals.

RESULTS

The participants who were considered "COVID-19-positive" were the ones who had either a positive PCR test or a positive serology test for COVID-19, totalizing 151 cases. The remaining group (N=860) was considered the "COVID-19-negative" group.

Concerning the course internships, a greater incidence of COVID-19 was found in residents when compared with other students (p<0.001). There was no difference in frequencies comparing residents and interns and also among different universities (Table 1).

The only symptom that showed significant difference between the two groups was dysgeusia or anosmia, which displayed higher incidence among the COVID-19-positive group, emphasizing their high specificity for COVID-19.

There was a greater prevalence of the virus by contact with a relative (father/mother/siblings/uncle/brother-in-law) or a patient (p=0.002) when compared with other types of contact (spouse/partner, colleague, classmates, or other). In regard to the practical activities with patients and to the workplace, the highest frequency of COVID-19 was detected in participants who were caring for patients (p=0.001). Moreover, the type of care associated with the highest risk of being infected took place in emergency care settings (p=0.007).

With respect to the exposure time frame, a higher incidence of infection in those participants with 3-month of contact and also in those with 6-month of contact was found. There was a minimal difference found concerning the number of shifts worked or the number of days worked. In terms of regularity of the contact, there was a higher incidence of participants with COVID-19 for those who had contact with patients from 4 to 6 times a week when compared with the group that did not have the virus.

The participants' contact with patients known to be infected by the SARS-Cov-2 was divided in terms of regularity: monthly (since March 2020) and weekly (in the last month). There was

Table 1. Absolute and relative frequency of the university and apprentice stage in relation to the COVID-19 group.

	COVID-19				
	Negative (n=860)		Positive (n=151)		
Variable	n	%	n	%	p*
Apprentice stage					<0.001
Undergraduate	502	58.4	62	41.1	
Intern	202	23.5	34	22.5	
Resident	156	18.1	55	36.4	

(*) Descriptive level of probability of the chi-squared test.

a statistical difference when the frequency of the contact was from 4 to 6 times a week per month year-round (p<0.001) or from 4 to 6 days a week in the last month (p<0.001; see Table 2).

Concerning the use of PPE, there was a lower incidence of infection with the use of goggles in outpatient facilities and greater incidence while using gloves and caps in hospital settings having COVID-19-positive group when compared with the COVID-19-negative group. There was a higher frequency of COVID-19 in respondents who did not use PPE when they were in contact with colleagues inside the workplace (p<0.001).

In Table 3, it was observed that subjects from 26 to 29 years of age and those over 30 years of age displayed 1.92 and 3.26 greater probability of contracting COVID-19, respectively, than the students between 18 and 22 years of age. The residents showed a probability 2.10 times greater than the undergraduate students of having COVID-19. Participants with activities in emergency care settings displayed a probability 1.74 times greater of having been infected by the virus than the ones without this type of activity. Undergraduate students and residents in contact with patients 2–3 times a week or for more than 4 days a week displayed 2.21 and 4.50 higher probability of contracting COVID-19 than the students whose contact was only once a week. Wearing goggles in outpatient facilities decreased the probability of having COVID-19 by 64% while the nonuse increased the chance of having the virus by 2.78 times. The participants who wore gloves and caps in hospitalization settings had, respectively, 2.57 and 3.22 greater probability of having the virus in relation to the ones who did not use these items. Finally, the participants who had contact with colleagues without using any PPE had a probability of contracting COVID-19 at a rate of 3.22 times greater than the ones who wore PPE in such situations.

Table 2. Absolute and relative frequency of the number of practical activities with patients who were knowingly infected, in relation to the COVID-19 group.

	COVID-19				
	Negative		Positive		
Variable	n	%	n	%	p*
Activities with infected patients					0.064
Yes	329	59.5	81	68.6	
No	224	40.5	37	31.4	
Weekly regularity of the contact					<0.001
Only once	52	16.0	6	7.5	
Total of 2–5 times	133	40.8	18	22.5	
Once a week	40	12.3	8	10.0	
2–3 days a week	51	15.6	16	20.0	
4–6 days a week	34	10.4	25	31.3	
Every day of the week	16	4.9	7	8.8	
Monthly regularity of the contact					<0.001
No contact	68	21.3	13	16.1	
Only once	76	23.8	11	13.6	
Total of 2–5 times	72	22.6	13	16.1	
Once a week	36	11.3	12	14.8	
2–3 days a week	40	12.5	11	13.6	
4–6 days a week	20	6.3	14	17.3	
Every day of the week	7	2.2	7	8.6	

(*) Descriptive level of probability of the chi-squared test.

The variables which presented some significance in the univariate analysis were selected for the study of the multivariate. Only variables measured in both groups (COVID-positive and COVID-negative) were considered. Through the use of a multivariate logistic model with the "stepwise" variable selection, it was demonstrated that the variables "regularity of contact" and "use of goggles in outpatient facilities (protection factor)" were the only ones that presented an independent association with the prevalence of positive test for COVID-19 (Table 3). The respondents with contact higher than 4 days a week had a chance of contracting COVID-19 3.43 times higher than those with contact at least once a week in the last month. The students who did not wear goggles in outpatient facilities displayed a chance of having COVID-19 4.35 times higher than the ones who wore this PPE.

DISCUSSION

The exposure of healthcare professionals to SARS-COV-2 is known to be greater than in the general population. The hospital work and the direct contact with infected patients are the major elements that contributed to the greater contamination rate observed in this group.

Some evidence suggests that students and younger doctors adopt fewer protective measures both in relation to the use of PPE and to the low adhesion to behavioral methods when they are compared with more experienced doctors[10]. In this study, the highest prevalence of cases with COVID-19 occurred in participants who were over 30 years of age. In contrast, the highest proportion of respondents without COVID-19 was in participants between 18 and 22 years of age. Residents are generally older than undergraduate students and are obviously more exposed due to heavier workload. This result was also expected probably because undergraduate students, generally younger, had no practical activities during a significant part of the pandemic.

Table 3. Odds ratio value and confidence interval (CI) to 95% of the univariate and multivariate models of the selected variables for the prediction of COVID-19.

	Univariate		Multivariate	
Variable	**Odds ratio (95%CI)**	**p**	**Odds ratio (95%CI)**	**p**
Age				
18–22 years old	1.00	–		
23–25 years old	1.34 (0.85; 2.12)	0.260		
26–29 years old	1.92 (1.18; 3.11)	0.008		
Over 30 years old	3.26 (1.88; 5.65)	<0.001		
Apprentice stage				
Undergraduate	1.00	–		
Internship	0.73 (0.45; 1.15)	0.177		
Residency	2.10 (1.30; 3.37)	0.002		
Activity in emergency care settings				
No	1.00	–		
Yes	1.74 (1.16; 2.59)	0.007		
Weekly regularity of contact				
Up to once a week	1.00	–	1.00	
2–3 days	2.21 (1.13; 4.32)	0.021	1.86 (0.87; 3.95)	0.109
4 days or more	4.50 (2.53; 8.02)	<0.001	3.43 (1.78; 6.60)	<0.001
Use of goggles in outpatient facilities				
No	1.00	–	1.00	–
Yes	0.36 (0.15; 0.85)	0.020	0.23 (0.07; 0.78)	0.018
Use of gloves in hospital settings				
No	1.00	–		
Yes	2.57 (1.45; 4.53)	0.001		
Use of caps in hospital settings				
No	1.00	–		
Yes	3.22 (1.63; 6.36)	<0.001		
Contact with colleagues without PPE				
No	1.00			
Yes	3.22 (1.52; 6.81)			

PPE: personal protective equipment.

Other countries have also evaluated the prevalence of contamination among healthcare professionals. Houlihan et al. evaluated 200 healthcare professionals, with a contraction rate of 44% and a higher tendency of contamination among participants younger than 30 years of age, which contradicts the results obtained in our study[11].

An important finding, which determined higher prevalence of COVID-19, is the recognition that the infected index-person was a direct relative or patient being cared for by the student/resident. Consequently, the contamination can occur at home or outside the hospital.

A systematic review carried out to assess the impact of COVID-19 in healthcare workers included 64 separate studies, concluding that not washing hands constantly, using PPE insufficient or inadequately, working longer hours, and working in high-risk areas were the independent risk factors for COVID-19 infection[12]. The present analysis found a greater occurrence of the virus in professionals who did not use PPE and/or in professionals who had contact with colleagues without using PPE. This shows the importance of keeping the protective equipment on at all times in hospital settings. In addition, there was a greater

incidence of infection in those who worked for 3 and 6 months if compared with the ones without the virus. The frequency of exposure was decisive (weekly and monthly contact) for the contamination, with a greater proportion of cases with infection in those with more contact (from 4 to 6 times per week).

In relation to the symptoms, the most significant finding is the association of the virus with anosmia which, in accordance with the current available literature, suggests that hyposmia/anosmia is the most predictive symptom for COVID-19. A study carried out in two maternity wards in London evaluated the IgG seropositivity in 200 healthcare professionals (anesthesiologists, obstetricians, and midwives). Seroconversion rate was 14.5% (29/200 – 95%CI 9.9–20.1). Among the symptomatic subjects, anosmia was the only symptom predictor of seroconversion ($p<0.001$)[13].

Our study has limitations. Although it is multicentric, it is restricted to the universities in the Porto Alegre area at Rio Grande do Sul. These results cannot be generalized to other universities in our or other states of the country, since the pandemic context can present geographical variations. Moreover, recall bias is a limitation of this study design. The COVID-19 diagnosis was based on the report of the participants and their positive tests (PCR-RT or serology) were not confirmed. These limitations do not invalidate the study, which should be regarded as an instrument to promote new hypotheses to be tested in investigations specifically designed for this purpose.

CONCLUSIONS

The prevalence of COVID-19 antibodies was higher in the studied sample than in the general population of Porto Alegre, Rio Grande do Sul. The prevalence of COVID-19 was higher among residents and interns than in undergraduate students. Contact with an infected relative outside the hospital was associated with a higher occurrence of contamination. Anosmia was the only symptom more prevalent in the COVID-19-positive group. Contact with patients without wearing goggles and higher weekly frequency of contact were independent factors associated with the infection by COVID-19.

MEMBER OF BRAZILIAN MULTICENTER UNIVERSITY STUDY GROUP ON PREVENTION AND COMBATING COVID-19

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AUTHORS' CONTRIBUTIONS

JFLN, JLN, GOA, FNL, JPNL, VMFM, DPK, LFM: Conceptualization. **JFLN, JNL, GSS, GOA, FNL, JPNL, VMFM, LFM, ADL, GPL, MEDC, TM, MPP:** Data curation. **JFLN, JNL, GSS, GOA:** Formal Analysis. **GSS, GOA, FNL, JPNL, VMFM, LFM, ADL, GPL, MEDC, TM, MPP:** Investigation. **JFLN, JLN, GSS, GOA, FNL, JPNL, VMFM, LFM, ADL, GPL:** Methodology. **JFLN, JNL:** Project administration. **JFLN, JLN, GSS, GOA, FNL, JPNL, VMFM, LFM, ADL, GPL, MEDC, TM, MPP, RR, AVP:** Resources, Software. **JFLN, JLN:** Supervision. **GSS, GOA:** Validation. **JFLN, JLN, GSS, GOA, FNL, JPNL, VMFM, DPK, LFM, ADL, GPL, MEDC, TM, MPP, RR, AVP:** Visualization. **JFLN, JNL, GSS, GOA, FNL, JPNL, VMFM, DPK, LFM, ADL, GPL, MEDC, MPP, TM, RR, AVP:** Writing – original draft. **JFLN:** Writing – review & editing.

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ORIGINAL ARTICLE

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Drug utilization study in neonatal intensive care unit at tertiary care hospital

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SUMMARY

OBJECTIVE: Neonates are more susceptible to drug interactions and adverse effects, and special care should be taken when prescribing medication to them. This study aimed to investigate drug usage in the neonatal intensive care unit of a tertiary care hospital.

METHODS: This prospective observational study was conducted on 98 patients at the Apollo tertiary care hospital (Bannerghatta, Bangalore, India) in a period of 6 months. The most common indications for neonatal intensive care unit admission, average number of drugs per patient, the most frequently used medication, distribution of patients based on the birth procedure, and possible drug interactions were collected from patient profiles.

RESULTS: Among the patients, 52% were males and 48% were females. Notably, 38% of patients were preterm, 60% were term, and only 2% were post-term. Also, 80.6% were born by cesarean section and 19.4% were born by normal vaginal delivery. The highest mean of drug use was in the patient of 1,000–1,500 g (8.06 per patient). Preterm was the most frequent indication for admission in neonatal intensive care unit, followed by hyperbilirubinemia and then respiratory distress syndrome. The most frequently used medication was vitamin K (99%) and antibiotics followed by dextrose. In different types of antibiotics, amikacin (41%), cefoperazone+sulbactam (35%), cephalosporin (1%), ceftriaxone (0.7%), and amoxicillin (0.3%) were commonly administered. There were some possible interactions, such as aminoglycoside with furosemide and calcium gluconate.

CONCLUSION: Premature birth and resulting low birth weight were the main reasons for drug prescription. High administration of antibiotics is probably an area of concern and should be seriously considered.

KEYWORDS: Drug utilization. NICU. Infant.

INTRODUCTION

The drug utilization studies are used as effective tools in drug administration by the physician and hospital regarding audit and cost analysis and can recognize rational drug administration. With the rise of new drugs in the market, variations of prescribing pattern and administration of drugs, new adverse effects, and the concerns regarding the cost of these drugs, the importance of drug utilization studies is becoming increasingly apparent[1,2].

The drug utilization studies apply epidemiological methods to study the clinical use of drugs in populations[3]. These studies in newborns may be used to identify the major therapeutic problems. Although rational drug therapy is important for all individuals being treated with drugs, it is of paramount importance for neonates. Therefore, it is necessary to analyze the pattern of drug use in neonatal units and to identify therapeutic classes that should be prioritized for future research[4,5].

A neonatal intensive care unit (NICU) is a highly specialized unit that provides high-quality skilled care to premature, low birth weight, or critically ill newborn. Apart from facilities for continuous clinical and biochemical monitoring and life support systems, the neonatal intensive care management involves the use of a wide range of medications with well-defined and specified therapeutic objectives[6]. Because of the immaturity of various organ functions, such as kidney, liver, and gastric motility, the neonates may show pharmacodynamics and pharmacokinetic variations. Furthermore, they have a rapidly changing body surface area and weight, a rapidly developing system of drug absorption, metabolism, and excretion, and an inability to communicate with the provider, making them more susceptible to adverse drug reactions and irrational drug use[6,7].

The most common reasons for NICU admission are prematurity, respiratory distress syndrome (RDS), sepsis or infection, hypoglycemia, perinatal depression, and maternal chorioamnionitis[8,9]. Fluid therapy, antibiotics, vitamin K, vitamin D, calcium, and heparin are among the most common medicine used in NICU[10,11].

There are few drug utilization studies on NICU admitted patients. Neonate is a group of patients who are more susceptible to drug interactions and adverse effects, and special care should be taken when prescribing medication to them. This study aimed to investigate drug usage in NICU of a tertiary care hospital and possible drug interactions.

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METHODS

This prospective observational study was conducted on 98 patients at the Apollo tertiary care hospital (Bannerghatta, Bangalore, India) in a period of 6 months (from December 2019 to May 2020). Patients of either gender, who aged below 6 months, and who were admitted to NICU were included in the study. All patients who were admitted other than NICU and neonates who were not receiving any medications other than vaccines were excluded from the study. Full details of the cases, including gender, age, diagnosis, and drug therapy, were noted in the data collection form. All the patients were monitored until they shifted from NICU. The most common indications for NICU admission, average number of drugs per patient, the most frequently used medication, distribution of patients based on the birth procedure, and possible drug interactions were collected from patient profiles. Gestational age was defined as follows: preterm: born before week 37 of pregnancy; term: born between weeks 37 and 40 of pregnancy; and post-term: born after week 40 of pregnancy. The drug–drug interactions were analyzed using www.drugs.com as the resource. The study was approved by the Institutional Ethics Committee – Biomedical Research ("IEC-BMR") of Apollo hospital. The data were analyzed using SPSS version 16.0. and Microsoft Excel. The results were expressed in terms of percentages and numbers.

RESULTS

Among 100 patients enrolled in the study, 48 were females and 52 were males. Out of 100 patients, 60 were term, 38 were preterm, and 2 were post-term. In all 100 patients, preterm patients were having the most hospital stay with a mean of 5.94 days, and post-term patients were having least hospital stay with a mean of 3.5 days. Based on birth weight, the highest mean of drug use was 8.06 in the group of 1,001–1,500 g, followed by 7.67 in the group of 1,501–2,000 g. The least mean of drug use was 4.96 in the group of ≥4,000 g (Table 1).

Out of 98 patients, 79 were born by vaginal delivery, and 19 were born by cesarean section. Among the patients, 54 were appropriate for gestation age (AGA), 42 were small for gestation age (SGA), and 2 were large for gestational age (LGA). In addition, in preterm patients, most of them were SGA and, in term patients, AGA patients were most frequent (Figure 1).

Table 1. Mean drug use in different birth weight groups.

Weight (g)	1,001–1,500	1,501–2,000	2,001–2,500	2,501–3,000	3,001–3,500	3,501–4,000	>4,000
Mean of drug use (number)	8.06	7.67	7.03	6.12	5.87	5.24	4.96

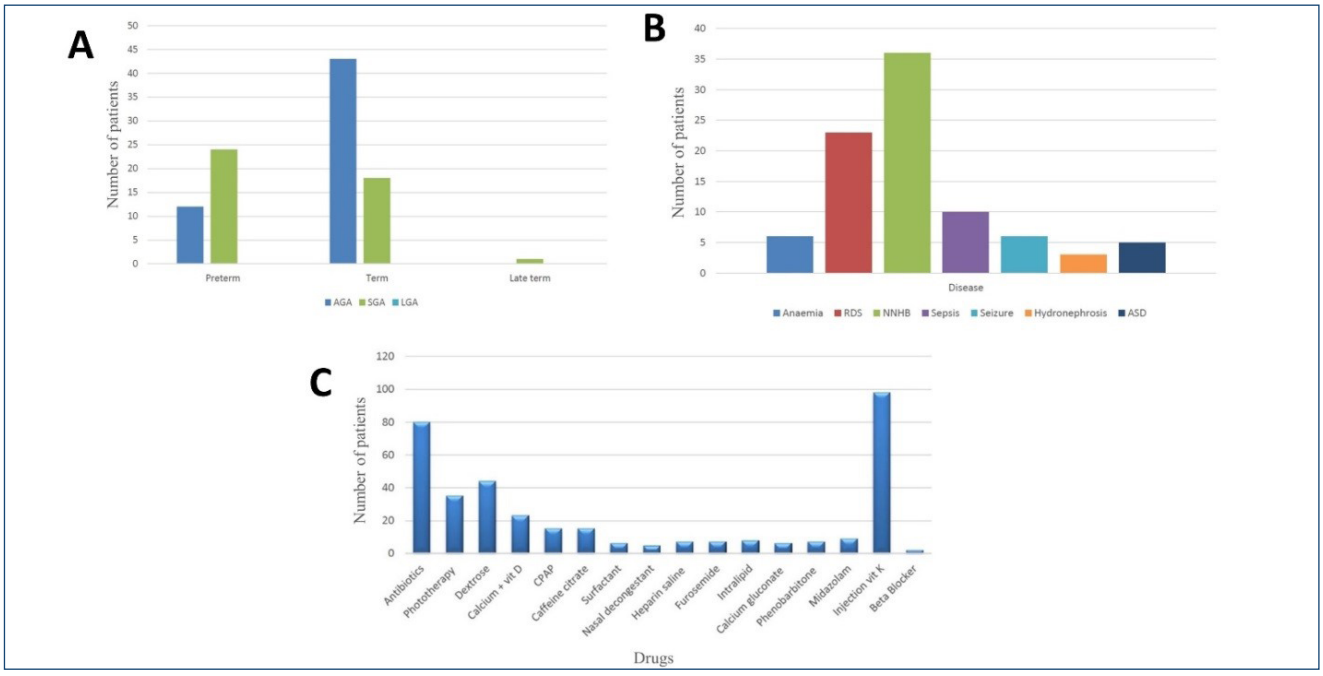

Figure 1. **(A)** Distribution of patients according to gestational age and birth weight. **(B)** Clinical indications for the neonatal intensive care unit admission and **(C)** different therapeutic class of drug prescribed. CPAP: continues positive airway pressure.

Analysis of the clinical indication for admission at the NICU showed that neonatal hyperbilirubinemia (NHB) (36%) followed by RDS (23 patients), sepsis (10 patients), anemia and sepsis (each 1 of the 6 patients), autism spectrum disorder (ASD) (5 patients), and hydronephrosis (3 patients) were the common clinical disorders (Figure 1B).

The most frequently used drugs in NICU were vitamin K and antibiotics [i.e., amikacin (41%), cefoperazone+sulbactam (35%), cephalosporin, ceftriaxone, and amoxicillin were the most commonly administered] followed by dextrose and phototherapy. Beta-blockers were the least frequently used drugs, followed by nasal decongestant (Figure 1C). Some possible drug interactions are also shown in Table 2.

DISCUSSION

Drug utilization study is an effective mechanism to identify individual variability in drug use and to promote interventions that will improve patient outcomes[12]. For the individual patient, the rational use of a drug implies the prescription of a well-documented drug at an optimal dose, together with the correct information, at an affordable price. Without the knowledge of how drugs are being prescribed and used, it is difficult to initiate a discussion on rational drug use or to suggest measures to improve prescribing habits. Information on the past performance of prescribers is the linchpin of any auditing system[1]. Neonates are a group of patients who are more susceptible to drug interactions and adverse drug reactions, and there are few studies on drug utilization pattern of these patients in NICU[4,13]. This was a prospective study in a tertiary care hospital which was conducted for a period of 6 months to investigate the utilization pattern of drugs in NICU.

Data indicated that 52% of patients were males and 48% were females similar to the results of Kumbhar et al.'s[14] (53.17% were males and 46.83% were females) and Choure et al.'s[15] (54.3% were males and 45% were females) studies. Among the patients, 38% were preterm, 60% were term, and only 2% were post-term. Unlike our results, Patel Brijal et al.[16] carried out a study on 650 neonates in the Government Medical College and Hospital, Rajkot, in which 59.85% were preterm and 38.46% were term. Regarding the birth weight of patients, 54% were AGA, 43% SGA, and only 2% were LGA, according to the study by Schlaudecker et al. Distribution of patients based on the birth procedure showed that 80.6% were born by cesarean section and 19.4% were born by normal vaginal delivery, which is comparable with the study by Gonçalves AC de S et al. (39% were vaginal delivery and 56.7% were cesarean section)[17].

The most frequent reasons for admission in NICU were preterm (39%), followed by hyperbilirubinemia (37%) and RDS (23%), which is contrary to the study by Patel Brijal et al. (32% sepsis, 27% RDS, and 25% meconium aspiration syndrome)[16]. These results indicate that prenatal care to prevent preterm delivery is one of the most important factors in reducing the rate of hospitalization and treatment costs.

Results showed that the average number of drugs per patient was 2.84. The most frequently used medication was vitamin K (99%) and antibiotics, followed by dextrose. The study by Neubert et al.[18] has reported lower administration of vitamin

Table 2. Different antibiotics used and possible drug interactions.

Antibiotics	Amikacin	Sulbactam+cefoperazone	Amoxicillin	Cephalosporin	Ceftriaxone
Frequency	33	28	3	10	6
Percentage	41	35	0.3	1	0.7

Possible drug interactions		
Drug 1	**Drug 2**	**Effect**
Aminoglycoside	Furosemide	Increase risk of nephrotoxicity and ototoxicity
Phenobarbitone	Triclofos (sedative)	Increase risk of sedation
Benzodiazepine	Triclofos (sedative)	Increase risk of sedation
Benzodiazepine	Aminoglycoside	Midazolam decrease effect of aminoglycoside
Dobutamine	Caffeine	Decrease sedation
Dobutamine	Furosemide	Decrease serum potassium
Phenobarbitone	Benzodiazepine	Decrease effect of benzodiazepine
Aminoglycoside	Calcium gluconate	Decrease calcium gluconate level
Benzodiazepine	Morphine	Increase sedation

K in neonates (90.06%). Choure et al.[15] conducted a study on 220 neonates in NICU of Government Medical College, Ambajogai, Maharashtra. Near to our results, the total number of drugs prescribed was 808, and the average number of drugs per prescription was 3.6. However, the most frequently prescribed therapeutic class of drugs were antimicrobial agents (60.64%), followed by vitamin K (26.7%) and aminophylline (9.4%). The average number of drug administration should be less as much as possible to reduce the chance of drug–drug interaction, and adverse effects of drugs, and the cost of treatment.

The results of this study showed that the infant's weight is a very influential factor in the amount of medication prescribed. Out of 98 patients, the highest mean of drug use was 8.06 and 7.67 in the patients of 1,000–1,500 g and 1,501–2,000 g, respectively, and it was reduced to 4.96 in the patients of >4,000 g. Consistent with our results, Kumbhar et al.[14] reported that the mean of drug use was 8.125 in ≤1,000 g birth weight neonates. Given that one of the main reasons for low birth weight is premature birth, prenatal care should be seriously considered.

In the present study, vitamin K was the most frequently used medication (99%), and antibiotics were the next followed by dextrose. The study of Neubert et al.[18] reported lower prescription of vitamin K in neonates (90.06%). Vitamin K deficiency bleeding is of particular concern in neonates as they are born with low levels of vitamin K. Current recommendations suggest single intramuscular administration of vitamin K as an effective, safe, and sustainable approach to prevent vitamin K deficiency bleeding.

Antibiotics were the second most prescribed drugs. In different types of antibiotics, amikacin and cefotaxime were the most commonly administered. Previous study by Warrier et al.[5] reported higher exposure to antibiotics like ampicillin (94.22%) and cefotaxime. In general, high numbers and doses of antibiotics in infants, especially because of the potential for antibiotic resistance, should be prescribed more carefully.

The current study had some limitations. It was conducted in a short period of time, and it would have been better if it had lasted up to a year to prevent the seasonal variation of disease pattern. Data were collected from only one institute, and multicentric studies in this subject are needed. The patient case sheets were used for data extraction, which were the secondary source of data, and there was a possibility of bias.

CONCLUSIONS

This was a prospective study in a tertiary care hospital which was conducted for a period of 6 months to observe the utilization pattern of drug in NICU. Preterm delivery was one of the most important factors in the rate of hospitalization and treatment costs. Premature birth and resulting low birth weight were the main reasons for drug prescription. High administration of antibiotics is probably an area of concern and should be seriously considered.

AUTHORS' CONTRIBUTIONS

FN, SR: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology. **EMS, NBSM:** Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Influence of small-group experiential learning of integrated traditional Chinese and Western medicine on the oral health knowledge, beliefs, and behaviors of elderly patients with diabetes

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SUMMARY

OBJECTIVE: This study aimed at the oral health problems of elderly patients with diabetes. A training course of integrated traditional Chinese and Western medicine was constructed, helping patients improve their oral health quality of life.

METHODS: A randomized controlled prospective experimental study was conducted. A total of 190 elderly patients were divided randomly into an observation group and a control group with 95 cases in each. The control group received regular health education, while the observation group was based on the control group to implement the integrated experiential learning of traditional Chinese and Western medicine in small groups. The oral health knowledge, attitude, behavior, and blood glucose control status along with the oral health quality of life of the two groups were compared before the intervention and at 3-month postintervention.

RESULTS: Three months after the intervention, the fasting blood glucose control and the 2-h postprandial blood glucose/glycosylated hemoglobin levels in the observation group were significantly better than in the control group, and the difference was statistically significant ($p<0.05$). The oral health quality of life in the observation group was significantly better than in the control group, and the difference was statistically significant ($p<0.05$).

CONCLUSION: The small-group experiential learning model of integrated Chinese and Western medicine can promote the transformation of knowledge–beliefs–behaviors in elderly patients with diabetes, which is conducive to controlling blood sugar levels and improving the quality of oral health.

KEYWORDS: Diabetes mellitus. Integrated traditional Chinese and Western medicine. Small-group experiential learning. Oral health knowledge. Oral health attitude. Oral health behavior. Blood sugar level. Glycosylated hemoglobin. Oral health quality of life.

INTRODUCTION

Diabetes is a common endocrine and metabolic disease. It causes a series of complications, such as glaucoma, diabetic neuropathy, cataracts, oral or skin infections, and periodontal disease[1,2], threatening the health and life of the patient and placing a burden (of which oral disease is one) on the patient's family and on wider society. Oral problems are chronic inflammatory diseases, of which periodontal disease is one of the most prevalent chronic infections among adults, affecting more than 22% of diabetics[3]. Traditional Chinese medicine (TCM) believes that there are many fire syndromes in patients with oral diseases; these can be divided into actual and virtual fire[4]. Elderly patients should be treated according to their different clinical symptoms and the characteristics of elderly people to reduce oral disease caused by diabetes. Western medicine believes that if the patient's own blood sugar level is not well controlled, it can result in various infections in the oral cavity very easily, such as periodontal disease and oral mucosal lesions. Studies have clarified the two-way relationship between diabetes and oral health. Several studies have shown that periodontal treatment can improve glycemic control, most likely by improving insulin sensitivity[5]. To improve the oral problems of elderly patients with diabetes, in addition to controlling blood sugar, it is necessary to reinforce health education on oral problems. The small-group education model is a patient-centered model that fully encompasses the characteristics of the autonomy, interaction, and mutual assistance of group health education and provides the advantages of faster and more interesting learning than is

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possible individually[6,7]. Experiential learning allows learners to understand and use certain skills more flexibly through their own experiences and observations[8]. Both forms strengthen the communication and cooperation between educators and patients.

To explore the effects of oral health education on the control of blood glucose, this study used small-group experiential learning to educate elderly patients with diabetes and oral disease by means of integrated Chinese and Western medicine self-care training and combined the concept of TCM syndrome differentiation with holistic nursing. The training improves patients' awareness of diabetic oral health, strengthens positive attitudes, changes negative behaviors, and improves the oral health of elderly patients with diabetes.

METHODS

Research objects

Using a convenient sampling method, the study selected 190 elderly patients with diabetes and oral disease who were treated in the Department of Endocrinology, Stomatology, and Integrated Traditional Chinese and Western Medicine in our hospital from February 2020 to January 2021. Using the random number table method, the study subjects were allocated to an observation group or a control group, each with 95 cases. The admission criteria were as follows: (1) age ≥60 years, (2) compliance with diabetes with a diagnosis time of >1 year, (3) hospital stay >3 days, and (4) informed consent. The exclusion criteria were as follows: (1) patients with oral diseases caused by trauma and other reasons, (2) patients with acute complications, and (3) patients with severe liver/kidney damage or cardiovascular/cerebrovascular diseases. There was no statistically significant difference in the general data between the two groups of patients. There were 95 patients in the intervention group, 4 (4.2%) of which were lost after 3 months, and there were 95 patients in the control group, 3 (3.2%) of which were lost after 3 months. Comparing the loss rate of the two groups, the difference was not statistically significant (χ^2=0.356, p>0.05).

Research methods

The review of the training course

In the control group, the traditional methods of delivering health education were performed. In the observation group, the elderly patients with diabetic oral diseases were trained in integrated Chinese and Western medicine self-care on the basis of Western medicine health education, and the characteristics of TCM were highlighted, including frequent gargling and checking of cheeks after every meal. In dietary conditioning, according to the logical relationship between food and medicine and the same treatment of food and medicine, patients with diabetes should consume light, cool, nutritious, high-quality protein and multifiber foods and avoid fat, sweet flavors, mellow wine, barbecues, and spicy stimulants. Attention should be paid to the temperature of food. It is advisable to consume food that is "hot without burning lips and cold without shaking teeth"[9]. In terms of daily lifestyle and health, daily lifestyle methods were introduced that adapt to the four seasons, combine the dynamic and static, and consider the moderate activity method of work and rest. Emotional adjustment recording involved explaining to patients the relationship between emotion and health, guiding self-relaxation, and suppressing seven different emotions that lead to disease[10-12].

Implementation and evaluation

The data were collected based on the oral health knowledge, attitude, behavior, and the oral health quality of life of the two groups of patients for 3 months after intervention. The fasting blood glucose levels and the 2-h postprandial blood glucose and glycosylated hemoglobin data of the two groups of patients were collected.

Research tools

The oral Health Knowledge, Attitude, and Behavior Questionnaire for Elderly Diabetic Patients[13] was used in our study. The higher the score, the better the patient's oral behavior. In addition, the higher the score, the worse the patient's oral health and oral quality of life for the Chinese version of the Oral Health Quality of Life Evaluation Index for the Elderly.

Statistical processing

The data were analyzed using SPSS 22.0 statistical software. Statistical methods used included descriptive statistics, t-tests, analysis of variance, and χ^2 tests. Enumeration data were expressed in the form of frequency, and measurement data were expressed using mean and standard deviation. p<0.05 was a statistically significant score.

RESULTS

Comparison of blood glucose control between the two groups of patients before and after intervention

The results showed no significant difference in the control of blood glucose (fasting blood glucose and 2-h postprandial blood

glucose/glycosylated hemoglobin levels) between the two groups before intervention (all were p>0.05). After the application of the intervention, the intervention group's blood glucose (fasting blood glucose and postprandial blood glucose) and 2-h blood glucose/glycosylated hemoglobin levels were better than the control group, and the differences were statistically significant (all were p<0.05) (see Table 1).

Comparison of oral health knowledge, attitudes, and behaviors between the two groups of patients before and after intervention

The results revealed that the oral health knowledge, attitude, and behavior scores of the two groups of patients before intervention were not statistically different (all were p>0.05). After intervention, the oral health knowledge, attitude, and behavior scores of the intervention group were higher than those of the control group. The differences were statistically significant (all were p<0.05) (see Table 2).

Comparison of oral health quality-of-life scores between the two groups of patients before and after intervention

The results revealed that after intervention, the oral health quality-of-life scores of patients in the intervention group were higher than those in the control group, and the difference between the two groups was statistically significant (all were p<0.05) (see Table 3).

Table 1. Comparison of blood glucose control between the two groups before and after intervention.

Group	Cases	Fasting blood glucose		2-h postprandial blood glucose		Glycated hemoglobin	
		Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
Control group	92	8.28±1.02	7.49±0.85	10.82±1.86	10.59±1.81	8.02±0.81	7.25±0.86
Intervention group	91	8.33±1.17	6.91±1.78	11.29±2.04	9.55±1.22	8.36±1.66	6.58±1.26
T		−0.306	2.830	−1.625	4.555	−1.776	4.171
p		>0.05	<0.05	>0.05	<0.05	>0.05	<0.05

Table 2. Comparison of oral health knowledge, attitude, and behavior scores between the two groups of patients before and after intervention.

Group	Cases	Knowledge		Attitude		Behavior	
		Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
Control group	92	3.40±3.58	3.78±3.34	20.80±9.71	21.62±8.50	40 (43.48%)	49 (53.26%)
Intervention group	91	2.92±3.65	5.86±2.75	20.30±9.07	24.20±5.21	36 (39.56%)	66 (72.53%)
t/□2		0.897[a]	−4.586[a]	0.357[a]	−2.470[a]	0.289[b]	7.273[b]
p		>0.05	<0.05	>0.05	<0.05	>0.05	<0.05

Note: [a]The t-value of two independent samples t-test; [b]The chi-square value.

Table 3. Comparison of oral health quality-of-life scores between the two groups before and after intervention.

Group	Cases	Before intervention	After intervention
Control group	92	21.14±12.08	19.41±13.27
Intervention group	91	21.51±11.64	15.79±10.12
T		−0.208	2.074
p		>0.05	<0.05

DISCUSSION

Small-group experiential learning with integrated traditional Chinese and Western medicine can improve patients' blood sugar control

The control group adopted the traditional health education model, and although the blood glucose control of the patients improved, it did not reach the ideal level. The intervention group implemented the small-group experiential learning program of integrated traditional Chinese and Western medicine. Both the fasting blood glucose and after-meal levels (2-h blood sugar and glycosylated hemoglobin) in this group dropped significantly. The results of our study were consistent with the results of previous studies. Health guidance and health education by dentists, physicians, and diabetes educators to enhance quality of life have been reported to be remarkably effective[14]. Goodson et al.[15] assessed salivary glucose concentrations and other oral factors, such as dental caries and gingivitis, in patients with diabetes. In this study, high salivary glucose was associated with a reduction in the overall number of bacteria in saliva and a change in bacterial frequency in 8,173 patients. In addition, Cortelli et al.[16] estimated the impact of gingivitis treatment on oral health-related quality of life (OHRQoL). This treatment improved the quality of life and highlighted the correlation between periodontal care and the individual's daily life. The reason is that small-group experiential learning of integrated Chinese and Western medicine rejects unilateral cramming-style teaching and focuses on the acceptance and emotional responses of elderly patients, allowing them to feel fully respected, understood, and accepted. Moreover, experiential learning emphasizes that in the process of mastering knowledge and skills, not only can people "know and act," they can also gain experience improvement from deep reflection[17]. All these factors enable patients to recognize diabetes correctly and help reduce blood sugar levels.

Small-group experiential learning with integrated traditional Chinese and Western medicine can improve patients' oral health knowledge, beliefs, and actions

Elderly patients with diabetes have poor oral health knowledge, beliefs, and behaviors, and they lack an understanding of the relationship between diabetes and periodontitis[18]. Li Yanling's research[19] shows that most people do not understand the close relationship between the two. Only after experiencing oral health problems, patients gain a certain understanding of this relationship. More than half of the patients have never received information about diabetes management and oral health from their healthcare providers, and preventive oral healthcare is not included in diabetes management programs. Research[20] shows that by communicating, preaching, and learning from each other's experiences, patients can help others to strengthen their beliefs, improve poor lifestyle habits, and develop good oral behaviors. An overseas survey revealed that more than half of the patients who visited a clinic for a year considered it unnecessary to undergo an outpatient oral examination[21], indicating that a firm belief in oral health had not been formed at that point. Surveys by Li Yanling and others show that more than half of elderly patients with diabetes lack oral health knowledge. The reason is that diabetes oral health education is rarely delivered in China; some of them are only at the level of traditional knowledge, and the content is not comprehensive. Consistent with the conclusions of other studies, the oral health education of elderly patients with diabetes adopts only a single form, and there is an urgent need to include multiple methods and channels of oral health education for elderly patients with diabetes to reduce the incidence of oral diseases.

Small-group experiential learning with integrated traditional Chinese and Western medicine can improve patients' oral health and quality of life

In recent years, with the transformation in medical models, people's concepts of health have developed from being historically disease free to have a good standard of all aspects of their own physical, psychological, and social activities. Therefore, introducing the "quality of life" evaluation index into the field of stomatology to evaluate the impact of oral diseases on patients' physical, psychological, and social functions is more suited to modern health perspectives[22]. As the course of the disease progresses in patients with diabetes, their oral quality of life gradually deteriorates[23]. Studies have shown that the oral health quality of life of elderly patients with diabetes in China is at a low-to-medium level. The impact of oral health problems, including periodontal disease, on the quality of life of elderly patients with diabetes mainly includes oral physiological functions, oral-related behavioral influences, cognitive problems, and psychological functions[24]. Health education can improve patients' awareness of diabetes and their knowledge of related oral health issues, enhance their oral healthcare capabilities, change their poor oral health behaviors, and improve their oral health quality of life[25].

Our study considered both the characteristics of the elderly and the educational content and implemented targeted small-group experiential learning of integrated Chinese and Western medicine in a scientific and effective new health education

model to assist elderly patients with diabetes in controlling their blood sugar levels. Stomatology, endocrinology, geriatrics, and other related departments must strengthen their collaboration to help elderly patients with diabetes develop good oral health knowledge, beliefs, and behaviors to achieve a decent oral health quality of life and to promote recovery[26].

CONCLUSION

This study integrates Chinese group experiential learning mode and promotes knowledge, belief and behavior changes in elderly diabetic patients, which helps to control blood sugar level and improve oral health quality. To achieve decent oral health, quality of life and promote rehabilitation.

ETHICS APPROVAL

This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Affiliated Hospital of Hebei University. Written informed consent was obtained from all participants.

AUTHORS' CONTRIBUTIONS

YW made substantial contributions to conception and design. **DXL, YLW** contributed for acquisition of data, analysis, and interpretation of data. **WT, JTW** were involved in drafting this manuscript and revising it critically for important intellectual content. **YMZ, LLL, YLL** gave final approval of the version to be published.

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ORIGINAL ARTICLE

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Prevalence of subhealth status and its effects on mental health and smartphone addiction: a cross-sectional study among Chinese medical students

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SUMMARY

OBJECTIVE: This study aimed to investigate the suboptimal health status or subhealth status and their relationship with mental health and smartphone addiction among Chinese medical students.

METHODS: A cross-sectional survey was conducted at Wannan Medical College of China in Wuhu.

RESULTS: A total of 2,741 students were surveyed in October 2020. Of 2,741 Chinese medical students who completed the survey, 904 (33%) participants reported to have had subhealth status. Anxiety status ($p<0.001$), depression status ($p<0.001$), and smartphone addiction status ($p<0.001$) have strong association with subhealth status.

CONCLUSION: This survey shows that the detection rate of subhealth status in Chinese medical students was 33%. Anxiety, depression, and smartphone addiction students had a higher detection rate of subhealth status. The anxiety, depression, and smartphone addiction of Chinese medical students are associated with subhealth status.

KEYWORDS: Health. Mental health. Students, Medical. China.

INTRODUCTION

In parallel with the development of social economy, there is a growing attention for the importance of health. The health status has been categorized into three different types, namely, health, disease, and the intermediate state between health and disease, called suboptimal health status or subhealth status (SHS). The SHS is considered as an intermediate state between health and overt disease. It is characterized by some disturbances in mental behaviors or physiological characteristics or in medical indexes but not typical pathological characteristics[1]. SHS is characterized by a decline in vitality, physiological function, and the adaptability to varying conditions. Suboptimal health is influenced by lifestyle and health awareness and can easily develop into chronic diseases, which is considered to be a subclinical and reversible stage of chronic disease. The prevalence of SHS in China is over 65% and has become a growing concern in many countries[2].

If SHS is handled properly, the body can transit to a healthy state, or vice versa, or turn into the disease state. It is pointed out that lifestyle behaviors were significantly associated with SHS[3]. More studies suggest that SHS is associated with mental health disorders. Depression and anxiety are considered the most common mental health disorders. Most of the previous surveys of subhealth have mainly focused on specific groups, such as teachers and civil servants. Studies have found that the speed, strength, and endurance of Chinese college students have demonstrated an overall decline since 2010[4].

The medicine is always recognized as one of the most stressful and demanding occupations. Due to heavy study loads and smartphone abuse, many college students, particularly medical students, do not get sufficient sleep time or exercise adequately. As a result, they may suffer from headaches, insomnia, fatigue, and/or forgetfulness. In addition, studies have pointed out that

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students had a high rate of poor health behavior practices, including poor dietary patterns, tobacco use, and sleeping habits[5]. But no research has been conducted to date on the relationship between the smartphone addiction and SHS among medical students. To explore the association between various lifestyle factors and suboptimal health conditions, we conducted a cross-sectional study among Chinese medical students. The current SHS of the Chinese medical students is poorly known. Therefore, it is very important to pay attention to the physical and mental health of medical students and the relationship between them.

METHODS

Participants and procedure

This cross-sectional study was conducted between October 14, 2020 and November 14, 2020 using a web-based questionnaire to analyze the influencing factors related to the SHS among medical college students at Wannan Medical College, China. In the beginning of each survey, questionnaire investigator introduced the purpose and nature of the questionnaire to the participants.

Research tools

Demographic characteristics

The basic sociodemographic information, such as gender, age, student's major, and academic year, were collected.

Suboptimal health status

Suboptimal health was evaluated by the Suboptimal Health Status Questionnaires-25 (SHSQ-25)[6]. SHSQ-25 consists of 25 items in total scored on a 5-point Likert scale (i.e., fatigue, cardiovascular health, digestive tract, immune system, and mental health). In the data analysis, "never or almost never" was assigned a score of 0, "occasionally" 1, "often" 2, "very often" 3, and "always" 4, providing a 0–100 total score. The higher the SHS score, the worse a participant's health status. Suboptimal health was defined in this study by a total SHS score ≥35. Cronbach's α for SHSQ-25 in this study was 0.83.

The Generalized Anxiety Disorder Scale-7

The scale comprises of seven items with 4-point frequency scale (0=not at all; 1=some of the time; 2=more than half the time; 3=nearly every day) in relation to the past 2 weeks[7]. Scores for 7 items were summed to obtain a Generalized Anxiety Disorder Scale-7 (GAD-7) total score, and the total GAD-7 score ranged from 0 to 21 points. In this study, anxiety symptoms was defined as a total score of GAD-7≥10.

Patient Health Questionnaire-9

This study used the Patient Health Questionnaire-9 (PHQ-9)[8] to assess the depression status of participants during the past 2 weeks. This scale contains a total of 9 items scored on a 4-point Likert scale (0=not at all; 1=some of the time; 2=more than half the time; 3=nearly every day). The total score ranged from 0 to 27, with a higher score indicating a higher risk for depression symptoms, and a total score of 10 points was defined as having depression symptoms.

Smartphone addiction

Smartphone addiction was assessed using the Smartphone Addiction Scale-Short Version (SAS-SV) designed by Kwon et al.[9]. SAS-SV is a widely validated questionnaire consisting of 10 items to assess the level of smartphone addiction during the last month. Each item is rated on a 6-point Likert-like scale ranging from "1=Strongly disagree" to "6=Strongly agree". The total score ranged from 10 to 60, and higher total score represents a higher level of smartphone addiction. The cutoff values for SAS-SV used for smartphone addiction were 31 in men and 33 in women.

Statistical analysis

The data were analyzed using IBM SPSS software version 20. Data are reported using mean±SD for continuous variables or as frequencies or percentages for categorical variables. A chi-square (χ^2) test was applied to compare between groups. The influencing factor of SHS was estimated using logistic regression analysis, odds ratio (OR), and their 95% confidence interval (CI). The two-tailed $p<0.05$ was considered statistically significant for all tests.

Ethical consideration

Ethical approval was approved by the Academic Ethics Committee of Wannan Medical College, Wuhu, China. All participants anonymously volunteered to participate in this study and signed an electronic informed consent form before filling out the questionnaire. Completing the electronic questionnaire was deemed consent.

RESULTS

Demographic characteristics

Characteristics of the participants are shown in Table 1. Of the respondents, 487 (17.8%) were freshmen, 786 (28.7%) were sophomore, 646 (23.6%) were juniors, and 822 (30.0%) were seniors. The majority of the participants (64.2%) resided

in urban areas. Participants' age ranged from 17 to 24 years with a mean age of 21.752±1.991.

The prevalence of subhealth status

As shown in Table 2, the prevalence of SHS was 33.0% (904/2741). There were significant differences on anxiety status (p<0.001), depression status (p<0.001), and smartphone addiction status (p<0.001), whereas no differences were found on age (p=0.388), academic year (p=0.388), area (p=0.109), whether class cadres (p=0.109), and love status (p=0.109). The prevalence of SHS by different variables is detailed in Table 2.

Binary logistic regression analyses of subhealth status

Table 3 displays the results of binary logistic regression analyses for SHS among medical college students. We found that anxiety (OR=2.991, 95%CI 2.285–3.915), depression (OR=4.697, 95%CI 3.468–6.362), and smartphone addiction (OR=3.375, 95%CI 2.728–4.176) were the risk factors for SHS.

Table 1. Sociodemographic characteristics of participating medical college students in the survey (n=2741).

Characteristics	Number	%
Age (years)		
≤20	1647	60.1
>20	1094	39.9
Academic year		
First	487	17.8
Second	786	28.7
Third	646	23.6
Fourth	822	30.0
Area		
Rural	1759	64.2
Towns	574	20.9
Cities	408	14.9
Class cadres		
No	1932	70.5
Yes	809	29.5
Only child		
No	1825	66.6
Yes	916	33.4
In love		
No	2059	75.1
Yes	682	24.9

DISCUSSION

Key findings

This study found that the overall detection rate of SHS was 33%, almost the same as the previous surveys[10], which indicates that the SHS occurrence among the medical students were ubiquitous. In late 2019, the COVID-19 pandemic became a serious health threat globally. Lifestyle is an important factor associated with SHS. Poor lifestyles include smoking, alcohol use, skipping breakfast, poor nutrition, lack of exercise, and sleep problems. It is pointed out that SHS was associated with chronic diseases and their development[11]. Due to the COVID-19 pandemic, people were restricted to stay at home for long periods, which leads to reduced physical activity, negative effects on physical health, and increased negative emotions[12].

Table 2. The detection rate of subhealth status in medical students using different variables (%).

Variable	Healthy	SHS	χ^2	p-value
Age (years)				
≤20	1114 (67.6)	533 (32.4)	0.715	0.398
>20	723 (66.1)	371 (33.9)		
Academic year				
First	321 (65.9)	166 (34.1)	1.064	0.786
Second	529 (67.3)	257 (32.7)		
Third	442 (68.4)	204 (31.6)		
Fourth	545 (66.3)	277 (33.7)		
Area				
Rural	1154 (65.6)	605 (34.4)	4.441	0.109
Towns	399 (69.5)	175 (30.5)		
Cities	284 (69.6)	124 (30.4)		
Class cadres				
No	66.5 (70.2)	648 (33.5)	0.928	0.335
Yes	553 (68.4)	256 (31.6)		
Smartphone addiction				
No	1103 (85.2)	191 (14.8)	368.148	<0.001
Yes	734 (50.7)	713 (49.3)		
Depression				
No	1139 (93.1)	84 (6.9)	681.231	<0.001
Yes	698 (46.0)	820 (54.0)		
Anxiety				
No	1235 (90.2)	134 (9.8)	665.570	<0.001
Yes	602 (43.9)	770 (56.1)		
In love				
No	1385 (67.3)	674 (32.7)	0.227	0.634
Yes	452 (66.3)	230 (33.7)		

Table 3. Binary logistic regression analysis for predictors of suboptimal health.

SHS-related factors	β	S.E.	Wald χ^2	p-value	OR	95%CI
Anxiety (no as a control)						
Yes	1.096	0.137	63.591	<0.001	2.991	2.285–3.915
Depression (no as a control)						
Yes	1.547	0.155	99.914	<0.001	4.697	3.468–6.362
Smartphone addiction (no as a control)						
Yes	1.216	0.109	125.393	<0.001	3.375	2.728–4.176
Constant	-3.514	0.140	626.598	<0.001	0.030	

In China, due to the learning pressure and smartphone abuse, the prevalence of SHS among medical students continues to rise. This study found that anxiety, depression, and smartphone addiction were correlated to low SHS scores. People who are always involved in surfing the Internet tend to have less face-to-face social interaction, less physical activity, and less sleep, all of which contribute to the development of subhealth symptoms.

The impact of subhealth status on mental health

This study revealed a significant association between SHS and anxiety and depression, with SHS associated with anxious and depressive symptoms. The COVID-19 pandemic has a significant impact on individual mental health. The most common distress responses include anxiety, depression, insomnia, fear of disease, and risky behaviors. The COVID-19 pandemic had created a lot of uncertainty. Due to the restrictions designed to prevent the spread of the virus, people began to experience financial problems, lost work, and often had to be isolated from their families, which made people prone to symptoms of depression and anxiety.

Additionally, sleep disturbances caused by psychological problems can increase the risk of inflammatory disorders and weakened immune system. Psychological problems, such as anxiety and depression, will also cause problems such as biological clock disorders, irregular eating, lack of sleep, and lack of physical exercise, and the accumulation of long-term problems will threaten the people's physical health, leading to subhealth. Anxiety and depression can lead to sleep disorders and increase individual fatigue, not conducive to physical recovery. When facing stressful environment, people with negative emotions are more inclined to choose smoking, alcoholism, or unhealthy diet and other harmful behaviors, which also aggravate subhealth conditions.

The impact of subhealth status on smartphone addiction

Since the popularity of smartphones began in 2007, smartphone ownership has grown steadily globally. As of 2020, the current number of smartphone users is 3.8 billion in 2021, which accounts for approximately 48.20% of the global population[13]. Moreover, today's college students grow up with the company of smartphones which become essential in their lives. Studies find that the prevalence of problematic smartphone use, including smartphone addiction, ranged between 10 and 30% among young people[14].

The impacts exerted by smartphone addiction on health are self-evident. Students addicted to smartphones will have adverse effects on their daily lives, such as difficulty in concentration, abnormal diets, reduction of productivity, broken social relationships, and health disorders such as blurred vision, neck stiffness, wrist pain, and sleep disorders. It is found that spending time more than 4 h a day online can significantly increase the risk of SHS[15]. As it is known, long-time surfing the Internet may cause backache, neck, finger, wrist, and arm pain, as well as anxiety, fatigue, and other SHS symptoms. Therefore, colleges should guide the medical students on physical exercise and psychological counseling in order to promote their physical and mental health development.

Limitations

The present study has some limitations. SHS is a subjective feeling which lacks objective clinical diagnostics. Social expectations or memory biases may arise due to participants' self-reported methods. Besides, the results were drawn from one medical college, hence these results cannot be simply generalized in all medical students.

ETHICAL APPROVAL

This study was approved by the Wannan Medical College Ethics Committee. Participants have been informed before answering the questions that participation in the survey was voluntary and anonymous and can be cancelled at any time, and that the results of the questions would be evaluated and published.

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AUTHORS' CONTRIBUTIONS

HL, MZ: Conceptualization. **HL:** Methodology. **ZZ:** Software. **LH, HL, MZ:** Validation. **LH:** Formal Analysis. **EZ:** Investigation. **MZ:** Resources. **HL:** Data curation. **HL, MZ:** Writing – original draft. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

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An easy and practical prognostic parameter: tumor–stroma ratio in Luminal, Her2, and triple-negative breast cancers

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SUMMARY

OBJECTIVE: The stroma surrounding the tumor cells is important in tumor progression and treatment resistance, besides the properties of tumor cells. Studies on the tumor stroma characteristics will contribute to the knowledge for new treatment approaches.

METHODS: A total of 363 breast cancer patients were evaluated for the tumor–stroma ratio. The percentage of stroma was visually assessed on hematoxylin–eosin stained slides. The cases of tumor–stroma ratio more than 50% were categorized as tumor–stroma ratio high, and those less than 50% and below were categorized as tumor–stroma ratio low.

RESULTS: Tumor–stroma ratio-high tumors had shorter overall survival (p=0.002). Disease-free survival tended to be shorter in tumor–stroma ratio-high tumors (p=0.082) compared with tumor–stroma ratio-low tumors. Tumor–stroma ratio was an independent prognostic parameter for the total group of patients (p=0.003) and also axillary lymph node metastasis and tumor–stroma ratio was statistically associated (p=0.004). Also, tumor–stroma ratio was an independent prognostic parameter in node-positive Luminal A and B subgroups for overall survival (p<0.001).

CONCLUSION: Tumor–stroma ratio is an independent prognostic parameter that can be evaluated quite easily in all molecular subtypes of all breast cancers and does not require extra cost and time to evaluate.

KEYWORDS: Stromal Tumor. Breast neoplasms. Survival. Prognosis.

INTRODUCTION

Globally, determining the biological behavior of breast carcinomas (BC), which are the most common cause of cancer deaths in women, is expected to produce essential knowledge for developing new therapeutic approaches[1]. Despite the ever-increasing knowledge accumulation, 30% of patients still develop recurrence after treatment. Therefore, it is thought that not only tumor cells, whose properties are well-known in many respects, but also the stroma surrounding the tumor cells are important in the progression and treatment resistance of the tumor[2,3]. It is a complex issue that how tumor stroma and its components follow in determining tumor behavior. It is thought that tumor stroma affects tumor progression by being affected by a cell to cell, cell to extracellular matrix, genetic, physiological, and environmental factors[4]. Studies on the characteristics of stroma surrounding the tumor cells will contribute to the knowledge for new treatment approaches[5].

Many different components contribute to tumor stroma. It is not always practical to evaluate these components separately, but even determining the tumor–stroma ratio (TSR) gives information about the prognosis of patients. It has been reported that the amount of tumor stroma is an independent prognostic parameter in many tumors[6-9]. In our study, the relation of TSR with prognostic parameters and survival was evaluated in both triple-negative (TN) and ER-positive BC. While the chemotherapy option of patients with TN and PT1/PT2 N0 is controversial, lymph node-positive patients are candidates for adjuvant chemotherapy[10]. We also investigated the role of TSR in survival in these node-positive Luminal A and B groups.

METHODS

Histopathological scoring

The TSR was visually evaluated as previously described by Mesker et al.[11]. The original 4 µm routine hematoxylin and eosin (H&E)-stained slides from formalin-fixed paraffin-embedded blocks of the primary tumor were assessed by conventional light microscopy (Olympus, BX-51, ocular 22 mm). The area with the highest tumor stroma was determined in the 4× objective. The most stroma-abundant area on the slide in which tumor

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cells can be seen on each side was scored using a 10× objective (north–east–southwest). The TSR was scored in multiples of 10 per image field (e.g., 10, 20, and 30%). The cases of TSR more than 50% were categorized as TSR high, and those less than 50% and below were categorized TSR low. Necrosis, in situ tumors, mucus-secreting tumor areas, previous biopsy areas, and peripheral sides of tumors were excluded in evaluating the TSR. Representative examples of microscopic fields selected for TSR quantification from TSR-high and TSR-low tumors are shown in Figures 1 and 2.

Evaluation of the TSR was assessed successfully in all the tumors (100%). Cohen's kappa coefficient revealed an almost perfect agreement in classification (kappa=0.85; 94% concordance in classification) for a set of tumors scored by both observers (ÇÖ and OO).

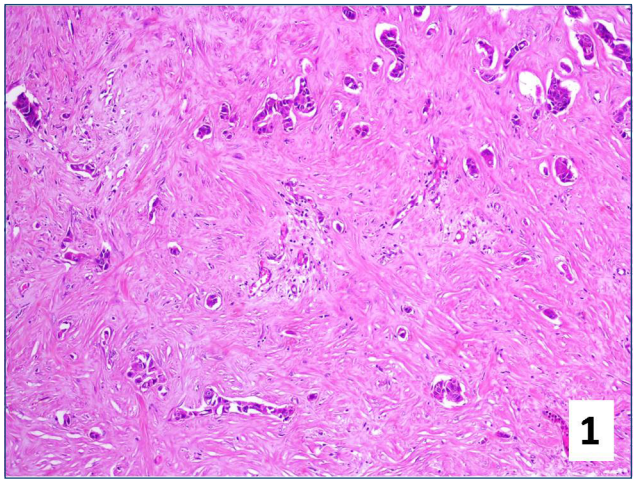

Figure 1. Example of stroma-rich (stroma ratio ≥50%). Hematoxylin and eosin-stained 4 µm sections of primary breast tumors (original magnification ×100).

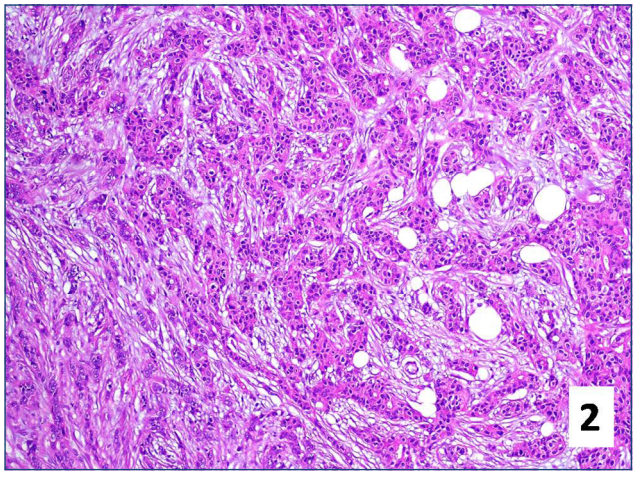

Figure 2. Example of stroma-poor (stroma ratio <50%). Hematoxylin and eosin-stained 4 µm sections of primary breast tumors (original magnification ×100).

Clinical and demographic data and tissues

We selected the patients with invasive breast cancer between 2010 and 2020 from the database of our hospital. Patients who received neoadjuvant chemoradiotherapy, who had distant organ metastasis at the time of diagnosis, who died due to post-op complications in the first month after surgery, whose clinical data could not be reached, who were not followed up after the operation in our hospital, whose hormone profile was not interpreted, who were out of follow-up for any reason, and whose HE slides and formalin-fixed paraffin-embedded blocks could not be found in our archive were excluded from the study.

The clinical information was obtained from retrieving the medical records, including gender, age, histological tumor type, grade, tumor size, lymph node status, type of surgery, and patient follow-up information. All cases were divided into molecular subtypes based on the ER, PR, HER2, and Ki-67 immunohistochemical staining patterns and histological types, according to the classification of breast cancer by World Health Organization[2,12].

As a result, 363 patients were included in the study, and the relationship between TSR, and clinicopathological parameters, disease-free (DFS), and overall survival (OS) were investigated.

Statistical analysis

Statistical analysis was performed using SPSS version 21 software. Kolmogorov–Smirnov test, histogram graphics, Mann–Whitney U test, chi-square, Fisher's exact test, Hosmer–Lemeshow test, log-rank test, Cox regression, and Kaplan–Meier survival analysis were performed. For statistical significance, the p-value was accepted as <0.05.

RESULTS

Tumor–stroma ratio and clinical and pathological parameters

Tumor–stroma ratio could be evaluated in a total of 363 patients, 2 of whom were men. The median age of patients was 55 (25–100) years, and the median follow-up time was 46 (2–132) months. A total of 324 of the cases had invasive ductal carcinoma (IDC), of whom 20 had invasive lobular carcinoma (ILC) and 19 patients had other histological types. When the cases were evaluated according to the T stages, 146 cases were found in T1, 196 cases were in T2, and 21 cases were found in the T3 stage. According to the molecular subtypes, 135 cases were Luminal A, 160 cases Luminal B, 30 cases Her2, and 32 cases were in the triple-negative group.

There was no statistical relationship between TSR and clinicopathological parameters such as age, hormone status, molecular

and histological type, and angiolymphatic invasion. Among the clinicopathological parameters, there was a statistically significant difference between axillary lymph node metastasis and TSR (p=0.004). Accordingly, as the TSR increased, the incidence of axillary lymph node metastasis increased. The main characteristics of the included studies are listed in Table 1.

Table 1. Correlations between tumor–stroma ratio and well-established prognostic factors.

		TSR low		TSR high		p
		N	%	N	Column N %	
Age	<57.2	104	61.50	101	52.10	0.069
	>57.2	65	38.50	93	47.90	
Histological types	IDC	149	88.20	175	90.20	0.594
	ILC	9	5.30	11	5.70	
	Others	11	6.50	8	4.10	
ER expression	ER negative	35	21.00	33	17.20	0.363
	ER positive	132	79.00	159	82.80	
PR expression	PR negative	54	32.30	52	27.10	0.277
	PR positive	113	67.70	140	72.90	
Ki67 expression	Low	54	37.00	70	44.00	0.211
	High	92	63.00	89	56.00	
Her2 expression	Her2 negative	110	65.90	122	63.20	0.361
	Her2 positive	32	19.20	48	24.90	
	Unknown	25	15.00	23	11.90	
Molecular subtypes	Luminal A	57	34.30	78	40.80	0.488
	Luminal B	76	45.80	84	44.00	
	HER2	15	9.00	15	7.90	
	Triple negative	18	10.80	14	7.30	
Nuclear grade	1	5	4.30	7	5.60	0.059
	2	84	73.00	104	83.20	
	3	26	22.60	14	11.20	
Histological grade	1	13	8.30	14	7.50	0.116
	2	109	69.40	146	78.50	
	3	35	22.30	26	14.00	
T stage	PT1	70	41.40	76	39.20	0.698
	PT2	91	53.80	105	54.10	
	PT3	8	4.70	13	6.70	
Perineural invasion	Negative	123	74.10	138	71.50	0.582
	Positive	43	25.90	55	28.50	
Anjiolymphatic invasion	Negative	96	56,80	91	46.90	0.06
	Positive	73	43.20	103	53.10	
Lymph node metastasis	Negative	104	61.50	90	46.40	0.004
	Positive	65	38.50	104	53.60	
Breast cancer-related death	Alive	159	94.10	160	82.50	0.001
	Dead	10	5.90	34	17.50	
Distant metastasis	Negative	152	89.90	161	83.90	0.089
	Positive	17	10.10	31	16.10	

Tumor–stroma ratio and prognostic associations with outcome

A total of 194 (54%) patients were classified as TSR high and 169 (46%) patients as TSR low. OS was found for patients with TSR-high tumors as compared to patients with TSR-low tumors in univariate analysis (p=0.003). In multivariate analysis, the TSR was an independent prognostic variable for OS (Table 2).

In univariate analyses (p=0.86), the TSR was not an independent prognostic variable for DFS. After correction for the factors also used in multivariate analyses for DFS, no significant difference was obtained.

Molecular subtypes and axillar lymph node metastasis were independent prognostic variables for DFS in multivariate analysis. Even performing Kaplan–Meier curve for OS showed a significant difference between TSR-high and TSR-low patients (p=0.002) (see Figure 3). A trend was seen toward a worse DFS for patients with TSR-high tumors compared to patients with TSR-low tumors in the Kaplan–Meier curve (p=0.082) (see Figure 4).

A total of 104 (61.5%) node-positive Luminal A and B breast carcinoma patients were classified as TSR high and 65 (38.5%) patients as TSR low. OS was found for these patients with TSR-high tumors as compared to patients with TSR-low tumors in univariate analysis (p:0.003). In multivariate analysis, the TSR was an independent prognostic variable for OS [Hazard ratio (HR) 5.33; 95%CI 1.224–23.203; p=0.026]. Patients with TSR-high node-positive Luminal A and B tumors show a significantly worse overall survival compared to patients with TSR-low tumors in the Kaplan–Meier curve(p<0.001) (see Figure 5). A trend was seen toward a worse DFS for patients with TSR-high tumors compared to patients with TSR-low tumors in the Kaplan–Meier curve (p=0.066) (see Figure 6).

Table 2. Cox univariate and multivariate analysis for overall survival of all patients.

Variables	Univariate	Multivariate	HR (95%CI)
	p	p	
Tumor–stroma ratio	0.003	0.042	2.381 (1.033–5.485)
Metastasis	<0.001	<0.001	7.038 (2.917–16.978)
ER expression	0.012		
PR expression	0.02		
Her2 expression	0.031		
Ki67 proliferation index	0.015		
Molecular subtypes	<0.001	0.001	10.382 (2.731–39.467)
Nuclear grade	0.223		
Histological grade	0.079		
Tumor size	0.013		
Age	<0.001	0.008	1.035 (1.009–1.062)
Lymph node metastasis	<0.001	0.025	3.945 (1.187–13.110)
Angiolymphatic invasion	<0.001		
Perineural invasion	0.375		

DISCUSSION

The relation of TSR with survival status was first investigated by Mesker et al. They found that patients with TSR more than 50% showed significantly worse OS and DFS. They suggested that TSR could serve as an independent parameter for predicting clinical outcomes in early-stage colon cancer[11].

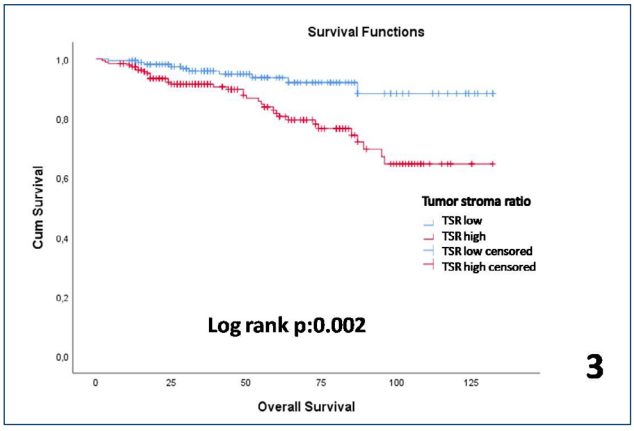

Figure 3. Kaplan–Meier curves for tumor–stroma ratio for the total patient population. Patients with TSR-high tumors show a significant overall survival compared to patients with TSR-low tumors.

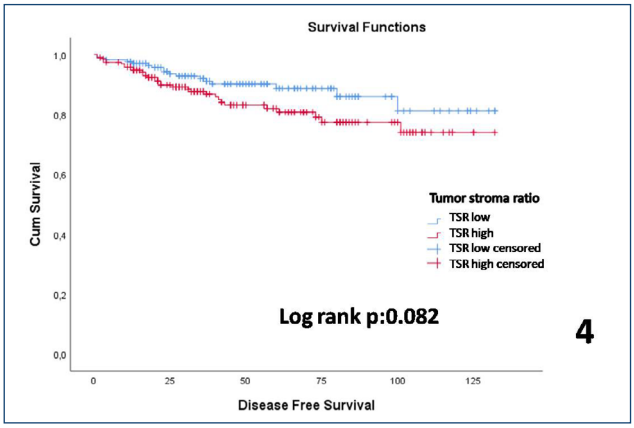

Figure 4. A trend was seen toward a worse disease free survival for patients with TSR-high tumors compared to patients with TSR-low tumors.

The tumor stroma consists of fibroblasts, pericytes, bone marrow-associated mesenchymal stem cells, adipocytes, macrophages, and immune cells[13]. These components play a role in neoangiogenesis, metastasis, and tumor progression[14]. It is not always morphologically possible to evaluate these components separately. Additional studies may be needed to evaluate these components; however, TSR can only be evaluated by light microscopy.

Routine pathology reports include the following parameters that affect patient prognosis, such as nuclear and histological grade, molecular subtype, lymph node metastasis, and lymphovascular invasion[15]. In addition to these parameters, the TSR, an independent prognostic indicator with many studies, is a convenient and valuable parameter for patient prognosis.

Studies in the literature evaluate stroma in BC using digital methods such as machine learning algorithms and automated analyses[9]. The advantage of digital methods over light microscopy is to evaluate whole tumor tissue with digital methods, while a more limited area is evaluated in light microscopy. While evaluating the stroma, the area of necrosis, the previous biopsy area, mucinous areas, and peripheral sides of tumors should not be evaluated. Therefore, the pathologist has a role in the selection of the appropriate tissue in digital analyzes. Digital methods are also not readily available, and extra costs are required to use them. For this reason, our study is based on visual eyeballing evaluation with light microscopy, which is very practical and does not require extra cost. Also, in our study, high intra-observer agreement kappa values for TSR prove strongly that TSR is a highly reproducible method.

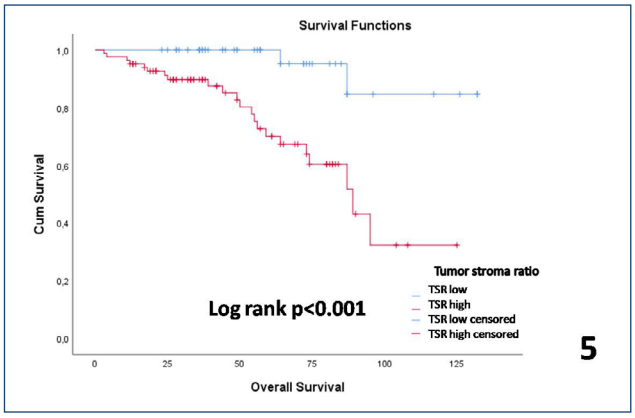

Figure 5. Kaplan–Meier curves for tumor–stroma ratio for node-positive Luminal A and B tumors. Patients with TSR-high tumors show a significant overall survival compared to patients with TSR-low tumors.

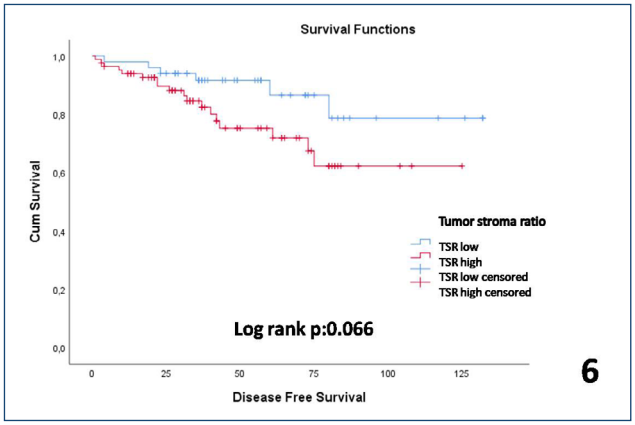

Figure 6. A trend was seen toward a worse disease free survival for patients with TSR-high tumors compared to patients with TSR-low tumors.

Recent studies have mainly worked on TSR of triple-negative breast cancers, which are negative for ER, PR, and HER2. Vangangelt et al. reported that a high amount of stroma predicts poor survival in TN BC. Kruijf et al. showed TSR to be an independent prognostic factor for DFS in breast cancer patients, especially in those with TN BC. Also, Dekker et al. confirmed this finding by a validation study in the EORTC peri-operative chemotherapy trial[18]. The relationship between molecular subtypes and TSR was evaluated in our study, but no statistically significant relationship was found (p>0.05). This result supports the idea that the molecular properties of tumor cells are independent of the molecular properties of the tumor stroma.

In this study, TSR was found to be associated with lymph node metastasis, which is a prognostic factor independent of clinicopathological parameters. The incidence of lymph node metastasis increases in patients with TSR high (p=0.004) compared to patients with TSR low. When the overall survival was evaluated according to TSR high and TSR low among 363 patients whose survival information was available, the OS of the group with TSR high was significantly shorter than TSR low (p=0.002) patients. Also, in univariate (p=0.003) and multivariate cox regression models, TSR was an independent prognostic variable for OS.

Tumor–stroma ratio was also examined in node-positive Luminal A and B groups to evaluate the relationship between TSR and survival in a more homogeneous group. The overall survival of this group with TSR high was significantly shorter than TSR low (p<0.001). Also, in univariate (p=0.003) and multivariate cox regression models, the TSR was an independent prognostic variable for OS. TSR is an independent risk factor in this group of patients whose survival may differ. It is a parameter that can be used to determine prognosis. A potential limitation of our study was that patients with a short follow-up period were also included in this study in order to evaluate more patients. This may be the reason why TSR and DFS are not associated.

Adjuvant chemotherapy is controversial in early-stage TN and ER-positive BC. 17th St. Gallen International Breast Cancer Conference suggested genomic assays in addition to

clinicopathological parameters in patient selection for treatment in PT1/PT2 N0 ER-positive patients[10]. However, in the same panel, it was reported that these genomic assays are not easily accessible universally and cost much more than routine pathology procedures. In our study, we showed that TSR is a decisive, independent prognostic factor. Therefore, TSR may be a parameter in the treatment decision, especially in this group of patients who have difficulties making treatment decisions.

There are publications in the literature reporting that clinical features are also effective in prognosis in BCs, regardless of the histological type[20-22]. One of the limitations of this study is that clinical features were not evaluated. Another limitation is that the molecular and histological types of the cases are not homogeneously distributed.

CONCLUSION

Tumor–stroma ratio is an independent prognostic parameter that can be evaluated quite easily in all molecular subtypes of all BCs and does not require extra cost and time to evaluate. Therefore, TSR is a candidate practical parameter that can be included in routine pathological reports.

ETHICS

This study was conducted at Recep Tayyip Erdogan University Research and Training Hospital, Rize, Turkey, and conducted in accordance with the Declaration of Helsinki. The Ethics Committee approved the study protocol of Recep Tayyip Erdogan University.

AUTHORS' CONTRIBUTIONS

ÇÖ, OO: Conceptualization, Investigation, Visualization, Project administration. **ÇÖ, OO, BŞ:** Data curation, Writing – orginal draft. **ÇÖ, BŞ:** Formal Analysis, Methodology. **RB:** Funding acquisition, Validation. **ÇÖ, RB:** Resources. **BŞ:** Software. **OO, RB:** Supervision. **OO, BŞ, RB:** Writing – review & editing.

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ORIGINAL ARTICLE

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Blood count and fasting blood glucose level in the assessment of prognosis and survival in advanced cervical cancer

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SUMMARY

OBJECTIVE: The objective of this study was to verify whether the parameters of the blood count and the fasting glucose level before treatment are related to prognosis and survival in cervical cancer (IIB–IVB staging).

METHODS: Patients with cervical cancer (stages IIB–IVB) were evaluated (n=80). Age, parity, staging, histological grade, histological type, hemoglobin, red blood cells, hematocrit, neutrophil, lymphocyte and platelet counts, red blood cell distribution width, neutrophil–lymphocyte ratio, platelet–lymphocyte ratio, fasting glucose levels, overall survival, and disease-free survival were evaluated. The results of laboratory parameters were compared using the Mann–Whitney U test. Receiver operating characteristic curve was used to obtain the area under the curve and determine the best cutoff values for each parameter. Survival was verified by using the Kaplan–Meier method, followed by the log-rank test. The level of significance was ≤0.05.

RESULTS: Regarding staging, lower hemoglobin values (p=0.0013), red blood cells (p=0.009), hematocrit (p=0.0016), higher leukocytes (p=0.0432), neutrophils (p=0.0176), platelets (p=0.0140), red blood cell distribution width (RDW) (p=0.0073), neutrophil–lymphocyte ratio (p=0.0039), platelet–lymphocyte ratio (p=0.0006), and fasting glucose level (p=0.0278) were found in IIIA–IVB compared with IIB staging. Shorter disease-free survival was associated with hemoglobin ≤12.3 g/dl (p=0.0491), hematocrit ≤38.5% (p=0.05), neutrophil–lymphocyte ratio >2.9 (p=0.0478), and platelet–lymphocyte ratio >184.9 (p=0.0207). Shorter overall survival was associated with hemoglobin ≤12.3 g/dl (p=0.0131), hematocrit ≤38.5% (p=0.0376), neutrophil–lymphocyte ratio >2.9 (p=0.0258), and platelet–lymphocyte ratio >184.9 (p=0.0038).

CONCLUSION: The analysis of these low-cost and easily accessible parameters could be a way to monitor patients in order to predict treatment failures and act as early as possible.

KEYWORDS: Uterine cervical neoplasms. Blood cell count. Glucose. Prognosis. Survival.

INTRODUCTION

Among the types of cancer with the highest incidence globally, cervical cancer occupies the eighth position in the world ranking. When analyzing only among the female population, it is the fourth type of cancer with the highest incidence and mortality[1]. In Brazil, cervical cancer remains the third position among the types of cancer with the highest incidence in the country, excluding the cases of nonmelanoma skin cancer[2]. One of the main factors related to the prognosis of cervical cancer is the staging, and detection through prevention programs is essential to increase survival[3].

Recent studies have shown that the systemic inflammatory response is related to factors of progression and prognosis in cervical cancer. Neutrophils and platelets are responsible for providing the bioactive substances necessary for molecules in neoplastic progression[4]. These bioactive agents are factors of angiogenic, epithelial, and stromal growth, as well as matrix remodeling enzymes. In addition, the imbalance in the innate and adaptive immune system will lead to lymphocytopenia and a compromised T-lymphocytic response. Consequently, the host's immune system response will not be fully effective[5].

The decrease in hemoglobin levels may occur with the progression of the disease. The values of the neutrophil–lymphocyte ratio (NLR) and the platelet–lymphocyte ratio (PLR) also have a potential use to be predictors of invasive cervical cancer, whose values tend to increase considerably in severe cases[6]. The value of NLR was also associated with the response to the chemoradiotherapy, and patients with higher NLR had a worse response[7].

Another change is related to the pretreatment fasting blood glucose level. The existence of an association between diabetes mellitus and gynecological cancer has been demonstrated[8]. Thus, the presence of pretreatment diabetes mellitus could be a parameter associated with worse prognosis and patient survival.

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The objective of this study was to verify whether the parameters of the blood count and the fasting glucose level before treatment in patients with cervical cancer in stages >IIB are prognostic and are related to survival.

METHODS

A retrospective study was carried out at the Department of Gynecology and Obstetrics (Oncology Research Institute – IPON) at the Federal University of Triângulo Mineiro (UFTM). Patients who underwent chemoradiotherapy treatment for cervical cancer (stages IIB–IVB; FIGO) were evaluated (n=80). The following medical record data were evaluated: age, parity, histological type, staging, neutrophil, lymphocyte and platelet counts, RDW, NLR, PLR, fasting glucose levels, number of deaths, overall survival (OS), and disease-free survival (DFS).

The inclusion criterion was patients with cervical cancer stages IIB–IVB. Exclusion criteria were immunosuppressive diseases and pregnancy. The prognostic factors evaluated were staging, histological grade, and histological type. DFS and OS were also assessed. The study was approved by the Research Ethics Committee of UFTM.

The data were analyzed using the GraphPad Prism 6 and MedCalc 19.0.4 software. The results of laboratory parameters were compared using the Mann–Whitney U test. For statistically significant parameters, a receiver operating characteristic (ROC) curve was used to obtain the area under the curve (AUC) and determine the best cutoff values for each parameter. Survival was verified by using the Kaplan–Meier method, followed by the log-rank test. The level of significance was ≤0.05.

RESULTS

The median age was 56 years (45–85), and the median parity was four births (0–14). The most frequent histological type was squamous cell carcinoma (86.25%), followed by adenocarcinoma (11.25%). Other histological types appeared in only 2.5% of cases. Regarding staging, 30 patients had stage IIB, and 50 patients had stages IIIA–IVB. The median, minimum, and maximum values of the laboratory parameters evaluated in stage IIB compared with stages IIIB–IVB are described in Table 1.

Regarding staging, lower hemoglobin values (p=0.001; medians 10.5 vs. 12.6), red blood cells (p=0.0014; medians 3.8 vs. 4.75), and hematocrit (p=0.0011; medians 33.6 vs. 37.65) were more associated with stages ≥IIIA–IVB than in stage IIB. On the leukogram, absolute values of leukocytes (p=0.0245; medians 9,280 vs. 7,655) and neutrophils (p=0.0088; medians 6,206 vs. 4,877) were higher in IIIA–IVB staging than in IIB staging.

When the platelets were evaluated, their absolute value was higher in IIIA–IVB staging than in IIB staging (p=0.0120; medians 324,000 vs. 258,000).

Evaluating RDW, NLR, and PLR, their values were higher in IIIA–IVB staging than in IIB staging (p=0.0047; medians 14.6 vs. 13.5; p=0.0022; medians 3.6 vs. 2.3; and p=0.0007; medians 191.1 vs. 127.6, respectively). Regarding fasting blood glucose level, their values were higher in IIIA–IVB staging than in IIB staging (p=0.0263; medians 102.8 vs. 94.65). There was no statistical significance when assessing the grade of differentiation and histological type.

Subsequently, ROC curves were constructed to verify the cutoff values. According to the cutoff values found, the

Table 1. Values of the laboratory parameters evaluated in stage IIB compared with stages IIIB–IVB (median, minimum, and maximum values).

	Stage IIB (n=30)	Stages IIIA–IVB (n=50)
Hemoglobin (g/dl)*	12.6 (7.6–16.6)	10.5 (6–15.2)
Red blood cells (× 10^6/mm^3)**	4.75 (2.9–5.5)	3.8 (2.13–5.36)
Hematocrit (%)***	37.65 (23.2–47)	33.6 (18.3–46.6)
Leukocit (/mm^3)$^+$	7,655 (4,750–16300)	9,280 (4,500–24,360)
Neutrophil (/mm^3)$^{++}$	4,877 (1,494–12,877)	6,206 (2,475–18,026)
Lymphocytes (/mm^3)	1,970 (1,179–3,927)	1,844 (115–3,167)
Platelets (×10^3/mm^3)$^{+++}$	258 (165–449)	324 (157–702)
RDW$^{\#}$	13.5 (12.2–19.6)	14.6 (12.3–21.4)
NLR$^{\#\#}$	2.3 (0.6–6.7)	3.6 (1.2–92.6)
PLR$^{\#\#\#}$	127.6 (68.1–317)	191.1 (77.1–1,852.2)
Fasting glucose level (mg/dl)$^{\#\#\#\#}$	94.65 (72.3–161)	102.8 (74.4–170.2)

Mann–Whitney test. *p=0.001; **p=0.0014; ***p=0.0011; $^+$0.0245; $^{++}$p=0.0088; $^{+++}$p=0.0120; $^{\#}$p=0.0047; $^{\#\#}$p=0.0022; $^{\#\#\#}$p=0.0007; $^{\#\#\#\#}$p=0.0263.

Kaplan–Meier curves were constructed to assess DFS and OS. Shorter DFS was associated with hemoglobin ≤12.3 g/dl (p=0.0491), NLR >2.9 (p=0.0478), and PLR >184.9 (p=0.0207). The parameters of hematocrit ≤38.5% (p=0.05) and red blood cells ≤4.38 million/mm^3 were shown at the significance threshold (p=0.0603) (Figure 1).

Shorter OS was associated with hemoglobin ≤12.3 g/dl (p=0.0163), hematocrit ≤38.5% (p=0.0451), NLR >2.9 (p=0.0305), and PLR >184.9 (p=0.0031). The parameter of red blood cells ≤4.38 million/mm^3 was shown at the significance threshold (p=0.0689) (Figure 2).

DISCUSSION

The relationship between anemia and prognosis has been demonstrated in several types of gynecological tumors[9-12]. In ovarian cancer, higher levels of platelets were found in advanced disease, since hemoglobin levels were higher in stages I/II[12]. In our study, lower hemoglobin values, red blood cells, and hematocrit were more associated with staging ≥IIIA–IVB than in IIB staging. Higher hemoglobin values before treatment for uterine cervical cancer were associated with longer DFS, but were not associated with OS in multivariate analysis[10]. We found an association of lower hemoglobin levels with both DFS and OS.

Leukocytosis, neutrophilia, thrombocytosis and lymphocytosis, as well as PLR and NRL are related to prognosis, stage of the disease, and response to the treatment of cervical cancer. Neutrophils secrete vascular endothelial growth factor, interleukin (IL)-18, and metalloproteinases, thus contributing to tumor growth, vascularization and metastasis. Neutrophilia can then promote tumor metabolism by secreting several proliferative factors[13]. One study analyzed blood count parameters in 110 patients with preneoplastic diseases and cervical cancer, and found significantly higher values of neutrophils in these patients[14].

In contrast, lymphocytes exert antitumor effects by inhibiting the proliferation and migration of tumor cells, promoting apoptosis and mediating cytotoxicity. Patients with lymphopenia imply immunosuppression and worse survival. Therefore, NLR may reflect a balance in the host's antitumor immunity[13]. The increase in neutrophils may also be related to neoplastic progression and may suppress lymphocyte antineoplastic properties. Patients with invasive cervical cancer have higher NLR and PLR compared with patients with preneoplastic lesions of the cervix. Significant correlations were also found between the PLR value and depth of stromal infiltration, tumor size, and lymph node metastases. The decrease in the number of lymphocytes has

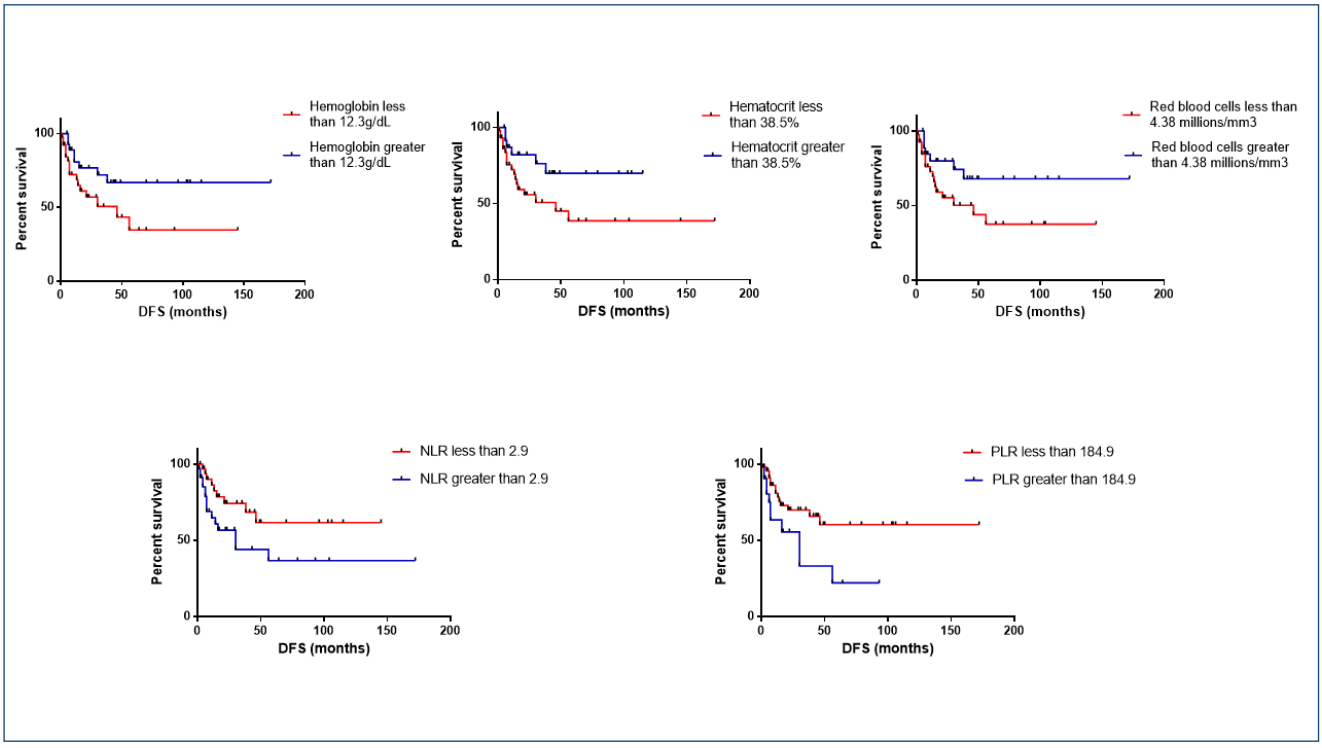

Figure 1. Disease-free survival: hemoglobin (p=0.0491), hematocrit (p=0.05), red blood cells (p=0.0603), neutrophil–lymphocyte ratio (p=0.0478), and platelet–lymphocyte ratio (p=0.0207).

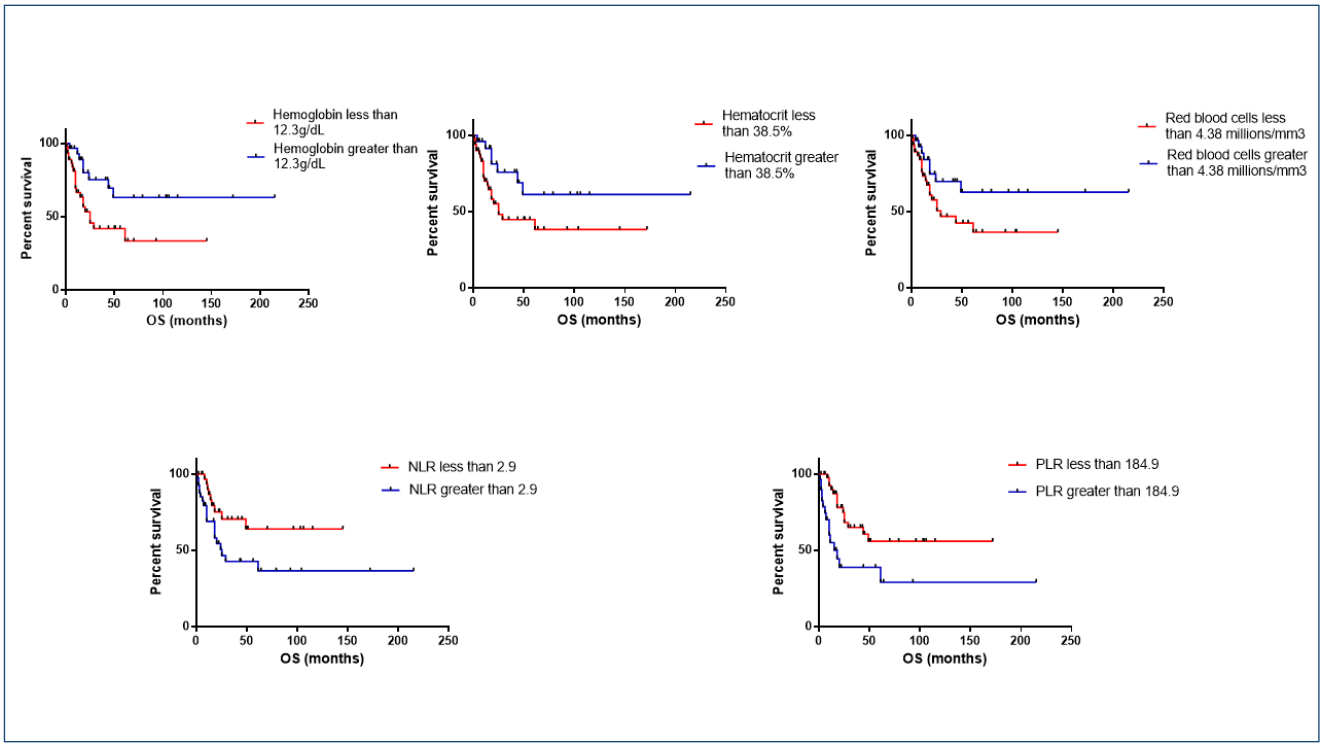

Figure 2. Overall survival: hemoglobin (p=0.0163), hematocrit (p=0.0451), red blood cells (p=0.0689), neutrophil–lymphocyte ratio (p=0.0305), and platelet–lymphocyte ratio (p=0.0031).

been demonstrated in more advanced stages of the disease[14]. Our study shows a significant association between NLR, disease staging, and patient survival. Higher NLR values were associated with more advanced disease staging and lower DFS and OS.

Platelet count is another marker of host systemic inflammation. Certain pro-inflammatory cytokines, such as IL-1, IL-3, and IL-6, stimulate thrombopoiesis in cancer patients. Platelets have also been implicated in processes that lead to angiogenesis in tumors by releasing vascular endothelial growth factor and other proangiogenic factors, including urokinase-type plasminogen activator. PLR may then be high in more advanced stages of cervical cancer[13]. Thrombocytosis may be associated with shorter survival and more advanced staging[6,15]. In our study, we found that PLR values >184.9×10³/mm³ are associated with shorter OS and DFS.

Regarding glucose, a study showed that patients with fasting glucose level ≥102 mg/dl in the pretreatment with chemoradiotherapy had a shorter survival compared with patients with fasting glucose level <102 mg/dl[16]. A retrospective study investigated the impact of glycemic control on survival and response to neoadjuvant chemotherapy in advanced cervical cancer and found that poor glycemic control was considered an independent predictor of survival and response to chemotherapy[17]. Our results demonstrated that fasting glucose values were higher in IIIA–IVB staging than in IIB staging, but no association was found with survival.

Increased RDW can be associated with several types of cancer[18-20]. In the literature, we found only one study that demonstrated a correlation between RDW and invasion of cervical cancer[21], and two studies that evaluated RDW in cervical cancer, but found no association with prognostic factors[22,23]. To the best of our knowledge, no study in the literature demonstrated the association of this parameter with cervical cancer staging. In our study, RDW values were higher in IIIA–IVB staging than in IIB staging.

CONCLUSIONS

The study of systemic laboratory parameters may lead to new discoveries of prognostic factors in patients with cervical cancer who have no surgical indication, and may better guide the oncologist for more aggressive chemotherapy and radiotherapy treatments in those patients with worse prognostic factors. These parameters can be obtained with simple blood tests (complete blood count and fasting glucose level), using low-cost and easily accessible methods.

AUTHORS' CONTRIBUITIONS

SDSM: Data curation, Methodology, Writing – original draft. **SSO:** Investigation, Methodology, Writing – original draft. **EFCM:** Conceptualization, Formal Analysis, Supervision, Validation, Writing – review & editing. **RN:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project Administration, Writing – review & editing, and guarantor.

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ORIGINAL ARTICLE

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Predictors of mortality in patients less than 50 years old with coronavirus disease 2019: a multicenter experience in Istanbul

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SUMMARY

OBJECTIVE: The objectives of this study were to identify predictors of mortality in young adult patients with coronavirus disease 2019 and to assess the link between blood type and mortality in those patients.

METHODS: This multicenter retrospective study, which was conducted in seven training and research hospitals in Istanbul, involved young adults who aged ≥18 and <50 years and hospitalized with coronavirus disease 2019.

RESULTS: Among 1,120 patients, confusion at admission (p<0.001) and oxygen saturation (p<0.001) were significantly predictive factors of mortality. Blood type O was significantly associated with mortality compared to those discharged from the hospital (p<0.001). Among co-morbidities, the most reliable predictive factors were cerebral vascular disease (p<0.001) and chronic renal failure (p=0.010). Among laboratory parameters, high C-reactive protein (p<0.001) and low albumin (p<0.001) levels were predictors of mortality in young adult patients with coronavirus disease 2019.

CONCLUSIONS: SpO_2 at admission was the best predictor of mortality in young adult patients with coronavirus disease 2019. The mortality rate was increased by cerebral vascular disease and chronic renal failure. Also, high C-reactive protein and low albumin levels were predictive factors of mortality. Moreover, blood type O was associated with a higher mortality rate than the other types.

KEYWORDS: Adult. COVID-19. Mortality.

INTRODUCTION

Background

Coronavirus disease 2019 (COVID-19), caused by the highly contagious severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was first identified in Wuhan, China, in December 2019, and it has become the pandemic of the last century. SARS-CoV-2 has infected around 100 million people, 2 million of whom have died[1].

In the early stages of the pandemic, it was thought that COVID-19 affected only older adults and killed only geriatric patients. Therefore, most COVID-19 studies involved patients of advanced age. Indeed, the mortality rate is higher in patients with chronic medical conditions, such as diabetes mellitus (DM) and hypertension (HT)[2]. Moreover, high D-dimer and ferritin levels are predictive factors of mortality[3]. During the early days of the pandemic, the relationship between ABO blood type and COVID-19 susceptibility and mortality was much discussed[4]. As the pandemic progressed, COVID-19 deaths occurred in younger patients[5]. However, few studies have examined predictive factors of mortality, including laboratory findings, in younger patients.

In this multicenter study, we examined predictors of mortality in terms of demographics, co-morbidities, and laboratory findings in young adult patients with COVID-19 and evaluated the link between blood type and mortality.

METHODS

Study design and setting

This multicenter, retrospective, observational study was carried out between March 20, 2020 and June 30, 2020. Data were

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collected from seven research and training hospitals in Istanbul, Turkey. This study enrolled young adult patients (aged ≥18 and <50 years) who were admitted to the pandemic ward or pandemic intensive care unit with signs of COVID-19 pneumonia on chest computed tomography (CT) or positive in polymerase chain reaction (PCR) test. Admission decisions were made according to the COVID-19 guidelines[6] of The Coronavirus Scientific Advisory Board of the General Directorate of Public Health, Turkish Ministry of Health. According to these guidelines, patients with mild–moderate pneumonia, those with mild–moderate pneumonia with poor prognostic factors in laboratory tests at admission, or patients with severe pneumonia are hospitalized.

Data collection

The total number of patients admitted to emergency departments of the centers during the study period was 513,168, among whom 34,304 were hospitalized for any diagnosis. We identified 1,629 young adult patients with COVID-19.

Data were collected by searching for U06.0 and U07.3 International Classification of Disease codes in the hospitals' automation systems. We assessed patients' demographics, vital signs at admission, complaints at admission, comorbidities, result of PCR test, blood type, laboratory parameters, hematological findings, and biochemical findings.

This study was approved by the Institutional Review Board of XXX Research and Training Hospital, Istanbul, Turkey (no. 2020-KSSH-1331 and clinicaltrials.gov ID: NCT04479137).

Statistical analysis

Statistical analysis was performed by using MedCalc Statistical Software version 19.4.1. Patient data are expressed as medians for normally distributed variables and as percentages for categorical variables. The normality of the distribution of continuous variables was examined using the Kolmogorov–Smirnov test. Between-group comparisons of normally distributed parameters were conducted using Student's t-test; the Mann–Whitney U-test was applied for non-normally distributed parameters. Categorical variables were analyzed using chi-square test or Fisher's test.

RESULTS

Data from 1,120 patients with COVID-19 were evaluated. Among them, 60.4% (n=677) were males with a median (quartiles) age of 42.0 (35.0–46.0) years. Of the patients, 88.6% were discharged (survivors) and 11.4% (n=128) were died (non-survivors). The distributions of demographic parameters, complaints at admission, and co-morbidities in survivors and non-survivors are shown in Table 1. There were significant differences between non-survivors and survivors in age, SpO_2 at admission, confusion, fatigue, HT, DM, cerebrovascular disease (CVD), and chronic renal failure (CRF) (p<0.001). Based on age categories, i.e., 18–29, 30–39, and 40–49 years, 69.5% (n=89) of non-survivors were in the age group of 40–49 years. Of the total deaths, 21.9% (n=28) were in the age group of 30–39 years, and 8.6% (n=11) were in the age group of 18–29 years. Among 489 patients, the frequency of blood type O was significantly higher among non-survivors (p=0.010). The distribution of blood groups by age and survival is shown in Figure 1.

Laboratory parameters at admission are listed in Table 1. The lymphocyte, thrombocyte, calcium, and albumin values were lower, and the lactate dehydrogenase (LDH), blood urea nitrogen (BUN), ferritin, D-dimer, and CRP levels were higher in non-survivors than in survivors. Univariate and multivariate regression analyses were performed to identify predictors of mortality (Table 2). Model 1 comprised age, vital signs at admission, and complaints at admission, model 2 added co-morbidities and blood type O, and model 3 added laboratory parameters. A multivariate regression analysis showed that confusion (p<0.001) and SpO_2 at admission (p<0.001) were reliable predictors of mortality in model 1. In model 2, CVD (p<0.001), CRF (p=0.010), and blood type O (p=0.014); and in model 3, platelet count (p=0.022), albumin (p<0.001), BUN (p=0.005), and CRP (p<0.001) were predictive factors of mortality in young adult patients with COVID-19.

DISCUSSION

In this large multicenter study, we evaluated the factors associated with mortality in young adult patients with COVID-19. In this study, instead of PCR, we used chest CT because of its higher sensitivity. All patients included in the study had chest CT findings indicative of COVID-19. The key findings were as follows: (1) age was associated with mortality, and the majority of non-survivors (69.5%) were 40–50 years of age. (2) Confusion at admission, SpO_2 at admission, CVD, and CRF were independently predictive factors of mortality. (3) Non-survivors had a higher rate of blood type O (43.9%) than survivors. (4) The albumin and CRP levels were predictive factors of mortality.

Advanced age is a predictor of mortality in patients with COVID-19[7]. We obtained similar results and the mortality rate increased as patients approached 50 years. In a study of patients with COVID-19 who aged 18–34 years, male sex was a risk

Table 1. Characteristics of the patients and laboratory results.

	Non-survivors (n=128)	Survivors (n=992)	p-value
Male [%(n)]	62.5 (80)	60.2 (597)	0.614
Age (years)	42.5±6.1	39.0±8.4	**<0.001**
Admission systolic blood pressure (mmHg)	125.0 (110.0–140.0)	127.0 (110.0–140.0)	0.295
Admission diastolic blood pressure (mmHg)	70.0 (63.2–80.0)	70.0 (65.0–80.0)	0.350
Admission oxygen saturation (%)	92.0 (88.0–95.0)	97.0 (95.0–98.0)	**<0.001**
Admission heart rate (BPM)	92.0 (78.0–109.0)	87.0 (76.0–92.0)	**0.004**
Admission fever (°C)	37.0 (36.0–37.5)	36.8 (36.5–37.5)	0.349
Cough [%(n)]	50.8 (63)	64.8 (593)	**0.002**
Fever [%(n)]	44.3 (54)	41.6 (364)	0.584
Nausea and vomiting [%(n)]	15.6 (19)	9.5 (85)	**0.038**
Impaired consciousness [%(n)]	70.5 (86)	29.4 (267)	**<0.001**
Sore throat [%(n)]	4.2 (5)	10.3 (92)	**0.032**
Weakness [%(n)]	43.8 (53)	27.4 (244)	**<0.001**
Chest pain [%(n)]	12.1 (15)	6.7 (59)	**0.030**
HT [%(n)]	22.6 (28)	9.4 (83)	**<0.001**
DM [%(n)]	21.8 (27)	10.2 (90)	**<0.001**
CAD [%(n)]	6.5 (8)	2.5 (22)	**0.015**
CVD [%(n)]	9.7 (12)	0.8 (7)	**<0.001**
COPD/Asthma [%(n)]	8.1 (10)	5.1 (45)	0.172
CRF [%(n)]	10.3 (12)	1.9 (15)	**<0.001**
WBC count ($\times10^3$ per μL)	5.85 (3.3–9.5)	6.25 (4.7–10.4)	0.668
Platelet count ($\times10^3$ per μL)	160.0 (119.0–204.0)	206.0 (164.0–239.0)	**<0.001**
Neutrophil count ($\times10^3$ per μL)	4.38 (2.5–6.4)	4.44 (3.3–8.1)	0.092
Lymphocyte count ($\times10^3$ per μL)	0.85 (0.5–1.1)	1.3 (1.0–1.9)	**<0.001**
BUN (mg/dL)	16.5 (11.2–35.4)	12.1 (9.3–15.4)	**<0.001**
LDH (U/L)	382.5 (280.7–528.2)	312.0 (235.0–366.0)	**<0.001**
Albumin (g/L)	30.0 (24.0–33.0)	36.0 (32.0–40.0)	**<0.001**
CRP (mg/L)	95.6 (55.1–203.6)	51.8 (27.1–83.6)	**<0.001**
Ferritin (ng/mL)	617.6 (371.9–1497.0)	283.3 (120.8–629.0)	**<0.001**
D-Dimer (mg/L)	1.64 (0.7–3.1)	0.78 (0.4–1.4)	**<0.001**
Calcium (mg/dL)	8.3 (7.7–8.4)	8.5 (8.0–8.9)	**<0.001**

Bold indicates significance at p<0.005. Fisher's exact test. Data are means±standard deviation, medians (quartiles), and percentages for categorical variables.

factor for mortality and ventilation[8]. In contrast, we found no significant gender difference between survivors and non-survivors. COVID-19 can cause severe lung pathology and reduce the SpO_2; indeed, SpO_2 at admission and HR were predictors of mortality, as reported by Sands et al[9].

The COVID-19 mortality rate is higher in patients with co-morbidities, such as DM and HT. Here, the mortality rate was significantly higher in patients with HT, DM, [cardiovascular disease (CAD)], CVD, and CRF; the latter two parameters were reliable predictors of mortality in the multivariate regression analysis. It is hypothesized that COVID-19 causes coagulopathy and thrombosis and, therefore, aggravates CVD[10].

Siepmann et al. reported that COVID-19 is more likely to occur and to be more severe in patients with CVD[11]. Together with our results, this shows that COVID-19 and coagulopathy frequently co-occur. In the multivariate regression

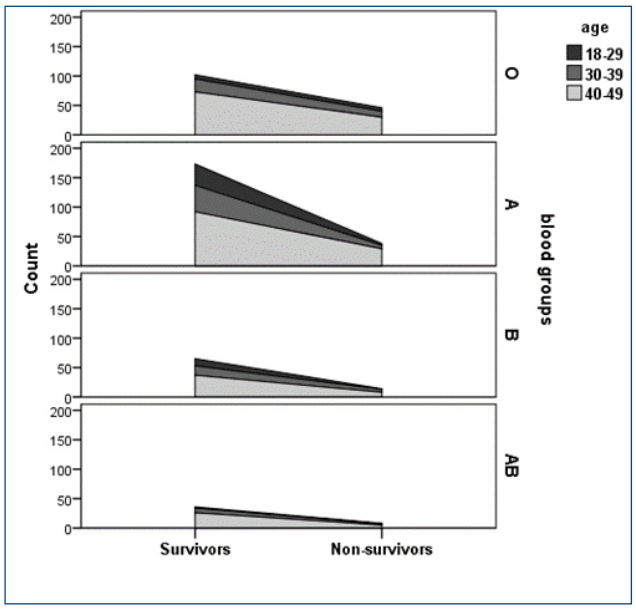

Figure 1. Distribution of blood groups by age and survival.

analysis, CRF was a more predictive factor of mortality than DM and HT. This study included 27 patients with CRF, 44.4% of whom died, suggesting that the mortality rate of COVID-19 is increased by CRF in younger patients. The International Society of Nephrology guidelines recommend that patients with CRF take stringent measures to prevent COVID-19[12].

The effect of ABO blood type and Rh factor on mortality has been debated. Zietz et al. reported that among 112 patients, the infection rate was higher for non-O types, and the mortality rate was higher for types AB and B compared with type O[13]. Similarly, Ray et al. reported that mortality from SARS-CoV-2 was lower among patients with blood type O[14]. Most studies of the relationship between COVID-19 severity and blood type concluded that the risk of severe disease is high for type A and low for type O[15]. Research on the relationship between COVID-19 and blood type is based on the similarity of genetic variants linked to the immune system and blood type[16]. In some studies in the literature, it was determined that there was no relationship between

Table 2. Regression analysis models for identifying predictors of mortality.

Variable	Univariate		Multivariate	
	OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value
Model 1				
Age (years)	1.051 (1.023–1.078)	**<0.001**	1.048 (1.011–1.087)	**0.011**
Admission heart rate (BPM)	1.007 (0.997–1.018)	0.170		
Cough	1.783 (1.223–2.600)	**0.003**	1.284 (0.753–2.190)	0.359
Impaired consciousness	36.583 (16.338–81.915)	**<0.001**	36.089 (10.925–119.212)	**<0.001**
Admission oxygen saturation (%)	0.736 (0.691–0.784)	**<0.001**	0.748 (0.697–0.802)	**<0.001**
Model 2				
DM	2.462 (1.525–3.974)	**<0.001**	1.428 (0.716–2.850)	0.312
HT	1.250 (0.348–4.486)	**<0.001**	1.672 (0.858–3.257)	0.131
KAH	2.702 (1.176–6.210)	**0.019**	1.225 (0.360–4.158)	0.745
CVD	13.408 (5.172–34.762)	**<0.001**	7.726 (2.167–27.554)	**<0.001**
CRF	5.862 (2.670–12.867)	**<0.001**	3.199 (1.317–7.771)	**0.010**
O blood group	2.084 (1.335–3.254)	**0.001**	1.847 (1.135–3.005)	**0.014**
Model 3				
Platelet count ($\times 10^3$ per μL)	0.994 (0.991–0.997)	**<0.001**	0.993 (0.986–0.999)	**0.022**
Lymphocyte count ($\times 10^3$ per μL)	0.745 (0.600–0.925)	**0.008**	1.375 (0.770–2.454)	0.281
Albumin (g/L)	0.963 (0.940–0.987)	**0.003**	0.060 (0.160–0.224)	**<0.001**
BUN (mg/dL)	1.015 (1.008–1.022)	**<0.001**	1.055 (1.016–1.094)	**0.005**
LDH (U/L)	1.007 (1.005–1.009)	**<0.001**	1.003 (1.000–1.006)	0.093
Calcium (mg/dL)	0.649 (0.487–0.866)	**0.003**	1.120 (0454–2.766)	0.805
CRP (mg/L)	1.001 (1.013–1.016)	**<0.001**	1.012 (1.006–1.019)	**<0.001**
Ferritin	1.134 (1.061–1.211)	**<0.001**	1.005 (0.926–1.090)	0.913
D-Dimer (mg/L)	1.362 (1.203–1.543)	**<0.001**	0.996 (0.893–1.112)	0.948

Bold indicates significance at p<0.005.

ABO blood group and COVID-19[5,17]. In contrast, Solmaz et al. reported that blood type O protects against COVID-19[18]. In this study, blood type O was associated with mortality among 489 patients and was a predictive factor of mortality in model 2.

Laboratory tests are typically performed to determine the severity of COVID-19. In Turkey, the Ministry of Health guidelines recommend considering the ferritin, lymphocytes, D-dimer, and CRP values when making decisions on hospitalization and treatment. Among middle-aged patients, a high D-dimer (>1 µg/mL) level and low albumin level were risk factors for severe COVID-19[6]. CRP, an acute-phase reactant, is an indicator of COVID-19 severity[19], as well as for other viral respiratory diseases, e.g., severe acute respiratory syndrome and Middle East respiratory syndrome[20,21]. In model 3, a high CRP level, hypoalbuminemia, thrombocytopenia, and a high BUN value were predictive factors of mortality, as well as of the severity of COVID-19 in young adult patients.

CONCLUSIONS

SpO_2 at admission is a reliable predictor of mortality in young adult patients who hospitalized with COVID-19 and so may be useful for triaging such patients at admission. Among co-morbidities, CVD and CRF were more predictive factors of mortality than DM and HT. In addition, blood type O, a high CRP level, and a low albumin level were predictive factors of mortality in young adult patients with COVID-19.

AUTHORS' CONTRIBUTIONS

RG: Conceptualization, Data curation. **ŞÇ:** Formal Analysis, Funding acquisition. **BGY:** Investigation, Methodology. **MÇ:** Project administration, Resources. **EA:** Software. **NMH:** Supervision, Validation. **GE:** Visualization. **İT:** Writing – original draft. **AÇ:** Writing – review & editing.

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ORIGINAL ARTICLE

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Respiratory muscle sequelae in young university students infected by coronavirus disease 2019: an observational study

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SUMMARY

BACKGROUND: The infection caused by coronavirus disease 2019 can lead to respiratory sequelae in individuals who have experienced severe or mild symptoms.

METHODS: An observational, cross-sectional study was developed, following the STROBE guidelines. Maximal inspiratory and expiratory mouth pressures were assessed in 50 healthy young students (26 women, 24 men; age 22.20±2.41 years). The inclusion criteria were as follows: aged between 18 and 35 years; control group: not diagnosed with coronavirus disease 2019; and coronavirus disease 2019 group: diagnosed with coronavirus disease 2019, at least 6 months ago. The exclusion criteria were as follows: obese/overweight; infected with coronavirus disease 2019 or coronavirus disease 2019 symptoms in the last 6 months; smokers; and asthmatics.

RESULTS: When comparing with groups, the coronavirus disease 2019 group presented statistically significant lower maximal inspiratory pressure values compared with the control group (88.32±16.62 vs. 101.01±17.42 cm H_2O; p=0.01). Regarding the maximal expiratory pressure, no significant differences were found. Similar results were found when performing a subgroup analysis by sex and group.

CONCLUSIONS: Young students who suffered from coronavirus disease 2019 asymptomatically or mildly at least 6 months ago presented a significant decrease in the inspiratory muscle strength as a sequel, so we believe that patients affected by this disease should have a brief postinfection assessment of this musculature to detect the indication for cardiorespiratory rehabilitation.

KEYWORDS: COVID-19. SARS-CoV-2. Students. Complications. Respiratory muscle.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, along with pending massive and effective vaccination globally, is challenging socioeconomic, health, and political systems. The virus enters the respiratory epithelium through the receptor for angiotensin-converting enzyme 2 (ACE2), causing respiratory infection and the well-known acute respiratory syndrome due to coronavirus disease (Sars-CoV-2)[1].

Sars-CoV-2 consists of an acute and sudden respiratory infection of variable course with fever, cough, dyspnea, anosmia, ageusia, muscle aches, diarrhea, chest pain, or headaches[2,3].

In 80% of cases, the symptoms are mild, while 14% present more severe forms with dyspnea, hypoxia, and pneumonia and 5% require admission to intensive care units (ICUs) with respiratory failure and multiorgan failure[4]. Comorbidities, such as obesity, hypertension, chronic obstructive pulmonary disease (COPD), or heart failure, increase mortality. Also, advanced age is the most relevant risk factor for the severity of the illness[5].

Between 40 and 45% of young population do not present symptoms[6]. Also, mortality associated with ages between 20 and 49 years is relatively low, which is around 0.0092%[7]. Even so, healthy young population without associated comorbidities are affected by COVID-19. Studies notify a possible genetic predisposition of these individuals[8].

The infection caused by COVID-19 can lead to respiratory sequelae, but not only in those individuals who have experienced severe forms. It seems increasingly clear that the sequelae are not related to the initial severity of the disease, and although patients who are treated in long-term intensive units suffer from post-ICU syndrome, many young people with mild initial involvement develop sequelae that last for weeks and even months[9].

The sequelae of COVID-19 widely vary, and their manifestations fluctuate between peaks of improvement and clinical worsening. COVID-19 is a multisystemic disease, so its sequelae are very diverse. Lopez-Leon et al. reported that 80% of people who have suffered from COVID-19 present persistent symptoms[7].

In a study carried out in Paris where symptoms were evaluated after an average of 111 days post-COVID-19 infection, the most prevalent symptoms were fatigue (55% of cases), headache (44%), dyspnea (42%), memory loss (34%), concentration and sleep disorders (28%), and hair loss (20%)[10].

Respiratory sequelae of COVID-19 infection are very common, and the most prevalent symptoms are pulmonary

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dysfunction (54% of cases), pleural thickening (27%), polypnea (21%), pain in the chest (16%), and pleural effusion (5%). The published data on persistent dyspnea are quite different, with the prevalence of being 8–43% at 4–8 weeks and 14% at 12 weeks[11].

Most of the data available so far suggest that 10–20% of patients affected by COVID-19 present symptoms 4 weeks after diagnosis. A study carried out in a sample of more than 4,000 people reported persistent symptoms in 13.3% of cases at 4 weeks, 4.5% at 8 weeks, and 2.3% at 12 weeks[12].

Regarding the sequelae described in the young population, the most prevalent symptoms are dyspnea on exertion and physical deconditioning. In this line, Crameri et al. published that VO2max had decreased by >10% in 19% of the 199 military personnel included in the study 45 days after COVID-19 diagnosis[13]. In another sample of 100 participants aged 45–53 years, regardless of the degree of severity of the infection, 60% of population had myocardial involvement and thus dyspnea on exertion[14].

Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) are adequate variables to assess respiratory function. MIP is the pressure generated during maximal inspiratory effort against a closed system. The MEP is measured during a similar maneuver with the total lung capacity[15].

COVID-19 patients who were admitted to ICUs and were assisted by mechanical ventilation later presented general and respiratory hypotonia[16]. However, concerning asymptomatic patients or with mild COVID-19 symptoms, no studies that report respiratory muscle dysfunction are available to date. Due to this reason, and due to the need to establish scientific evidence regarding this new disease, this study aims to assess respiratory muscle function in young patients who have been infected by COVID-19 in a mild or asymptomatic way.

METHODS

Study design

An observational, cross-sectional study was developed, following the STROBE guidelines from March to April 2021 at the Universidad Europea de Madrid.

Settings and participants

A total of 50 healthy young students were recruited at the Universidad Europea de Madrid (26 women, 24 men; age 22.20±2.41 years, height 172.01±7.47 cm, body mass 64.12±8.57 kg). Participants were recruited via email between February and March 2021. They were reassured that nonparticipation had no consequences. A code was assigned to participants before statistical analysis, thus guaranteeing the confidentiality of their data.

The inclusion criteria were as follows: (1) being a student at the Universidad Europea de Madrid; (2) aged between 18 and 35 years; (3) control group (CG): not diagnosed with COVID-19; and (4) COVID-19 group (COVID-G): diagnosed with COVID-19, with a positive polymerase chain reaction (PCR) test, at least 6 months ago.

The exclusion criteria were as follows: (1) obese or overweight; (2) infected with COVID-19 in the last 6 months; (3) smokers; (4) asthmatics; and (5) experienced COVID-19 symptoms in the last 6 months.

Ethical considerations

The current study respected the Declaration of Helsinki ethical statements throughout the study. All the participants read and signed the informed consent form before being part of this investigation.

Measurements

Maximal inspiratory and expiratory mouth pressures (MIP/MEP) were assessed using the Micro Respiratory Pressure Meter (FS985; Micro Medical, Los Angeles, CA, USA). These variables were measured in 25 subjects diagnosed with COVID-19 at least 6 months ago with mild symptoms or asymptomatic, and the same procedure was performed on 25 subjects who had not been ever diagnosed with COVID-19 or had experienced its symptoms.

The Sociedad Española de Neumología y Cirugía Torácica (SEPAR) 2003 procedures manual was followed. The participants rested for 5 min before performing the first maneuver. Then, they performed the maneuvers in a sitting position with a stuffy nose through a clamp that prevented air leaks and a straight back. The examiner showed the maneuver before its performance.

Participants started with MEP: an inspiration was requested at the maximum inspiratory volume with 1 s in inspiratory apnea and then exhaled as hard as possible. The participants rested for 1 min and then repeated the maneuver six times.

Next, MIP was performed. The participants were asked to exhale until the lung was empty, held for 1 s on maximum exhalation, and inhaled as hard as possible. They rested for 1 min between maneuvers until the six maneuvers recommended by the SEPAR were performed.

We recorded the highest value of the MIP and the highest value of the MEP, expressed in centimeter of H_2O.

Regarding anthropometric variables, height (cm; Ano Sayol SL height rod, Barcelona, Spain) and weight (kg; Asimed T2

scale, Barcelona, Spain) were measured. Then, by dividing the weight in kilogram by the height in meters squared, the body mass index (BMI, in kg/m^2) was calculated.

Statistical analysis

A descriptive analysis was developed for all the subjects using mean±standard deviation (SD) to describe the continuous variables. The Shapiro–Wilk test for the normality of the sample was conducted. For nonparametric variables, the Mann–Whitney U test was conducted, while the independent samples t-test was employed to compare the COVID-G with the CG and to determine differences between sex and the remaining of the continuous variables (i.e., MIP, MEP, BMI, age, weight, and height). The significance level was set at alpha <0.017 as three comparisons were performed[17]. All analyses were performed using SPSS version 27.0 statistical software.

RESULTS

Sociodemographic data of the sample

In total, 50 participants (26 women and 24 men), subjects (n=25) in the COVID-G, and subjects (n=25) in the CG were participated in the study. In both groups, 52% of participants were women, and 48% were men. The mean age of the COVID-G was 23.11±2.67 years, the body weight was 67.42±8.77 kg, the height was 174.03±8.07 cm, and the BMI was 22.21±1.50 kg/m^2. The mean age of the CG was 21.32±1.75 years, the weight was 60.80±7.08 kg, the height was 170.04±6.24 cm, and the BMI was 21.13±1.48 kg/m^2. In all these variables, there were significant differences between the two groups, with age, height, weight, and BMI being higher in the COVID-G compared with the CG.

Maximal inspiratory and expiratory mouth pressures

When comparing by groups, the COVID-G presented statistically significant lower values in the MIP, compared with the CG (88.32±16.62 vs. 101.01±17.42 cm H_2O; p=0.01). Regarding the MEP, no significant differences were found between the COVID-G and the CG (105.02±20.41 vs. 103.01±15.83 cm H_2O; p=0.64) (Table 1).

When comparing women by group, there were no statistically significant differences in age, height, weight, and BMI between the COVID-G and the CG. MIP values were lower, with a significant difference, in the COVID-G compared with the CG (75.21±11.60 vs. 88.72±10.61 cm H_2O; p<0.01). Regarding the MEP, no significant differences were found (91.81±20.10 vs. 92.30±11.23 cm H_2O; p=0.94) (Table 1).

Table 1. Subgroup analysis of the maximal inspiratory and expiratory pressures.

Variables	COVID vs. CG	Women: COVID-G vs. CG	Men: COVID-G vs. CG
MIP (cm H_2O)	88.32±16.62 vs. 101.01±17.42*	75.21±11.60 vs. 88.72±10.61*	103.12±5.45 vs. 114.03±13.60*
MEP (cm H_2O)	105.02±20.41 vs. 103.01±15.83	91.81±20.10 vs. 92.30±11.23	120.01±4.42 vs. 114.03±11.61

MIP: maximal inspiratory pressure; MEP: maximal expiratory pressure; COVID-G: COVID-19 group; CG: control group. *Significance was set at p<0.017.

When comparing men by group, the COVID-G had a higher height, weight, BMI, and age than the CG, with a statistically significant difference between the variables. MIP values were lower, with a significant difference, in the COVID-G compared with the CG (103.12±5.45 vs. 114.03±13.60 cm H_2O; p=0.016). No significant differences were found in the MEP (120.01±4.42 vs. 114.03±11.61 cm H_2O; p=0.13) (Table1).

DISCUSSION

In this study, the respiratory muscle strength was analyzed in young university students who had COVID-19 mildly or asymptomatically at least 6 months ago. Significantly lower MIP values were observed in the COVID-G compared with the CG, but no significant differences were found in the MEP values.

The COVID-G had significantly higher age, weight, height, and BMI values. Therefore, according to the predictive equations[18,19], the COVID-G should have presented higher MIP and MEP values. As it was not the case, it may be that the COVID-19 infection caused an impairment in the strength of the inspiratory muscles. Nevertheless, in a recent systematic review about predictive MIP and MEP mouth equations, they conclude that there is high heterogeneity in these equations, and none is reliable enough[20].

Comparing by groups and sex, the women of the COVID-G presented lower MIP values than those of the CG, with a statistically significant difference, without finding differences in the MEP. There were no significant differences between the women in both groups in terms of anthropometric variables. Probably, COVID-19 infection caused an impairment in the strength of the inspiratory muscles. Likewise, the men of the COVID-G presented lower MIP values than those of the CG, with a statistically significant difference, despite having a higher weight, age, height, and BMI. No differences were found regarding MEP values.

In patients with respiratory disease, a difference of 7–13 cm H_2O in MIP value is considered clinically significant[21]. In our case, although the population of our study is healthy, the COVID-G subjects presented a similar difference in MIP (being –13.51 cm H_2O in the case of women, or –15%; and –10.91 cm H_2O in the case of men, or –9.5%).

The principal sequelae of COVID-19 infection in the young population described in the literature are VO2max decrease (19% of cases)[13] and myocardial inflammation (60% of cases)[14]. These clinical situations are not correlated with the initial severity of the infection. In our study population, young people between the ages of 18 and 35 years, who had suffered from COVID-19 asymptomatically or mildly at least 6 months ago, presented a clinically significant decrease in inspiratory force[21], a fact that can be related to myocarditis or physical deconditioning, due to the relationship between MIP and VO2max[22].

Since 40–45% of young people affected by SARS-CoV-2 do not present symptoms[6], and the mortality associated with ages between 20 and 49 years is low, around 0.0092%, this population tends to relax barrier measures, has little fear of contagion, and postinfection monitoring is not usually performed. However, the data from this study suggest that young people who have been infected by COVID-19 should undergo an assessment of MIP and MEP, and in case of affectation, they should carry out specific respiratory rehabilitation.

Study limitations and future lines

As a limitation of this study, we could not compare the MIP and MEP values of our population with other reference values using predictive equations, since there are currently none with sufficient reliability[20].

CONCLUSIONS

Although the population of this study is young and experienced an infection by COVID-19 asymptomatically or mildly at least 6 months ago, we found a significant decrease in the inspiratory muscle strength as a sequel. All the patients affected by this disease should have a brief postinfection assessment of this musculature to detect the indication for cardio-respiratory rehabilitation and describe possible sequelae of Sars-Cov-2.

ETHICAL CONSIDERATIONS

The current study was approved by the Research Ethics Committee of Universidad Europea de Madrid and respected the Declaration of Helsinki ethical statements throughout the study. All the participants read and signed the informed consent form before participating in this investigation.

AUTHORS' CONTRIBUTIONS

GGPS: Investigation, Methodology, Formal Analysis, writing – original draft. **MPSF:** Data curation, Investigation, Project administration, Methodology. All authors actively contributed to the discussion of the results in the study and reviewed and approved the final version to be released.

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ORIGINAL ARTICLE

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The epicardial fat thickness is associated with fragmented QRS in patients with newly diagnosed metabolic syndrome

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SUMMARY

OBJECTIVE: The metabolic syndrome involves both metabolic and cardiovascular risk factors and is associated with cardiovascular mortality. Epicardial fat tissue plays a crucial role in deleterious effects of metabolic syndrome on the heart, including myocardial fibrosis. The fragmented QRS reflects heterogeneous depolarization of the myocardium and occurs as a result of fibrosis. Thus, we aimed to investigate whether there is an association between fragmented QRS and epicardial fat tissue in patients with metabolic syndrome.

METHODS: This study enrolled 140 metabolic syndrome patients, of whom 35 patients with fragmented QRS (+) and 105 patients with fragmented QRS (−). The two groups were compared with respect to clinical, laboratory, electrocardiographic, and echocardiographic indexes.

RESULTS: Fragmented QRS (+) patients had higher waist circumference, red cell distribution width, creatinine, left ventricular end-systolic diameter, left atrium diameter, septal a velocity, QRS duration, and epicardial fat tissue compared with fragmented QRS (−) patients. Waist circumference, red cell distribution width, QRS duration, left ventricular end-systolic diameter, left atrium diameter, septal a velocity, and epicardial fat tissue were significantly associated with the presence of fragmented QRS. The QRS duration and epicardial fat tissue were independently associated with the presence of fragmented QRS on surface electrocardiographic in metabolic syndrome patients.

CONCLUSIONS: Epicardial fat tissue and QRS duration were independently associated with the presence of fragmented QRS. Basic echocardiographic and electrocardiographic parameters might be used for the risk stratification in metabolic syndrome patients.

KEYWORDS: Electrocardiography. Echocardiography. Metabolic syndrome.

INTRODUCTION

The metabolic syndrome (MetS), a clustering of metabolic and cardiovascular risk factors, contributes considerably to cardiovascular mortality[1]. Visceral obesity seems to play a key role in the development of all features of MetS[2]. Epicardial fat tissue (EFT) is in direct contact with the myocardium, and it is very active metabolically. Recent studies have suggested that EFT with both local and systemic effects has an important role in deleterious effects of MetS on the heart[3,4]. In addition to its association with atherosclerosis, hypertension, and diabetes, new data suggest that MetS is also strongly associated with cardiac arrhythmias[5]. The MetS and EFT appear to associate with multiple electrocardiographic (ECG) abnormalities and cardiac arrhythmias[6]. In this context, previous studies have shown the association of EFT with ECG abnormalities, such as atrial fibrillation (AF), P-wave dispersion, QRS prolongation, QT prolongation, left axis deviation, ST-T wave abnormalities, and extrasystoles in patients with MetS[7,8]. Recent interest has focused on fragmented QRS (fQRS) as a novel resting ECG parameter. The fQRS, defined as the presence of R' wave or notching of R or S patterns with or without Q waves on a 12-lead ECG, has been shown to reflect heterogeneous depolarization of the ventricular myocardium that can occur due to ischemia, fibrosis, or scar[9,10]. Although a recently published study has shown that fQRS is associated with left ventricular dysfunction in patients with MetS[11], to the best of our knowledge, there is no study showing the relationship between fQRS, visceral fat, and MetS. In the present study, we examined the association of fQRS with EFT in newly diagnosed MetS subjects.

METHODS

Study population

MetS was diagnosed based on the concomitant presence of three or more risk factors established by the NCEP ATP III 2005 guidelines: systolic blood pressure (SBP) and diastolic blood pressure (DBP) ≥130 and ≥85 mmHg, respectively, fasting plasma glucose ≥100 mg/dL, waist circumference (WC) >102 cm for men and >88 cm for women, fasting triglycerides >150 mg/dL, and high-density lipoprotein cholesterol <40 mg/dL for men and <50 mg/dL for women[12]. Notably, 140 patients with newly diagnosed MetS constituted the final study population.

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A history of coronary artery or valvular heart disease, systolic heart failure (left ventricular ejection fraction <50%), diabetes mellitus, chronic liver or renal disease, hypothyroidism or hyperthyroidism, use of antihypertensive drug, statin, regular alcohol intake, drug abuses, QRS duration >120 ms, and incomplete or complete right and left bundle branch block were excluded. The Ethics Committee of Health Science University Van Education and Research Hospital approved the protocol.

Electrocradiography

Standard 12-lead surface resting ECGs (filter range, 0.5–150 Hz, 25 mm/s, 10 mm/mV) were recorded for all the study population. Heart rate (HR) and QRS duration were noted. The fQRS was defined by the presence of various RSR' patterns (QRS duration <120 ms) with or without Q wave, which includes an additional R wave (R') or notching of the R wave or S wave, or the presence of more than one R' fragmentation without typical bundle branch block in two contiguous leads[13]. The standard 12-lead ECG was analyzed without using any magnification.

Echocardiography

Standard parasternal and apical views were obtained in the left lateral decubitus position by using a Vivid 3 Pro ultrasound machine (GE Vingmed Ultrasound, Milwaukee, WI, USA). The left ventricular ejection fraction (EF), interventricular septum thickness (IVST), and posterior wall thickness (PWT) were measured on M-mode traces recorded in the parasternal long-axis view. Mitral inflow was assessed from the apical four-chambered view with pulsed-wave Doppler by placing a 1–2 mm sample volume between the tips of the mitral leaflets during diastole. EFT was measured according to the previously described method[2]. EFT was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium at end-diastole in three cardiac cycles. The maximum EFT was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aortic annulus. For the midventricular parasternal short-axis assessment, maximum EFT was measured on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the interventricular septum at midchordal and the tip of the papillary muscle level, as the anatomic landmark. The maximum value at any site was measured and the average value was calculated. Measurements were done according to established standards[14].

Statistical analysis

Continuous variables were presented as mean±standard deviation or median [interquartile range (IQR)], and categorical variables were expressed as number and percentage. The Kolmogorov–Smirnov test was used to identify the normally distributed variables. The continuous variables were compared across the groups using Student's t-test or the Mann–Whitney U test. The categorical variables were compared using the chi-square test. The correlation between fQRS and EFT was calculated using point-biserial correlation analysis. To determine the independent predictors of fQRS, multivariable logistic regression analysis was performed. Due to the small number of dependent variables (number of fQRS(+) patients=35), we calculated Firth's penalized likelihood bias reduction in logistic regression analysis to avoid overfitting using R-software version 3.6.3 (R statistical software, Institute for Statistics and Mathematics, Vienna, Austria). Variables that were found statistically significant in the univariate analysis were entered in the multivariable logistic regression model. All data were analyzed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

RESULTS

The comparison of demographic, biochemical, clinical, ECG, and echocardiographic parameters is shown in Table 1. Weight, WC, red cell distribution width (RDW), rate of insulin use, and creatinine were significantly higher in Group 1 compared with Group 2. QRS (105 vs. 86 ms, p<0.001), LA (41 vs. 39 mm, p=0.001), left ventricular end-systolic diameter (LVESD) (29 vs. 28 mm, p=0.022), septal a velocity (11 vs. 10, p=0.011), DT (200 vs. 180, p=0.038), and EFT (9.96±1.38 mm vs. 7.34±1.03 mm, p<0.001) were significantly higher in Group 1 compared with Group 2. EFT was significantly correlated with fQRS (r=0.556, p<0.001) (Figure 1). WC, RDW, QRS, LVESD, LA, septal a velocity, and EFT were significantly associated with the presence of fQRS in univariable logistic regression analysis. Multivariable logistic regression analysis with variables that exhibit statistical significance in univariable regression showed that only QRS duration [odds ratio (OR)=1.166, p<0.001] and EFT (OR=3.441, p=0.002) were independent predictors of the presence of fQRS (Table 2).

DISCUSSION

In this study, fQRS(+) patients had higher EFT and QRS duration compared with fQRS(–) patients. Furthermore, EFT and

Table 1. Demographic, clinical, laboratory, electrocardiographic, and echocardiographic indexes between fragmented QRS(+) and fragmented QRS(−) metabolic syndrome patients.

Variables	fQRS(+) (n=35)	fQRS(−) (n=105)	p-value
Age, years)	56±7.54	56±7.56	0.985
Gender, n (%)			0.077
Male	20 (57.1)	42 (40)	
Female	15 (42.9)	63 (60)	
BMI, kg/m^2	33.2 (30.3–39.3)	32.4 (29.8–35.1)	0.092
Height, m	1.66±0.09	1.63±0.07	0.13
Weight, kg	90 (86–105)	86 (81–90)	0.031
Waist circumference, cm	105 (95–115)	98 (92–104)	0.014
Systolic BP, mmHg	140 (140–160)	140 (135–150)	0.218
Diastolic BP, mmHg	85 (85–90)	85 (85–90)	0.119
Diabetes mellitus, n (%)	33 (94.3)	88 (83.8)	0.157
Hypertension, n (%)	31 (88.6)	95 (90.5)	0.749
CAD, n (%)	6 (17.1)	15 (14.3)	0.682
History of familial CAD, n (%)	10 (28.6)	32 (30.5)	0.831
Cigarette smoking, n (%)	16 (45.7)	40 (38.1)	0.426
CHF, n (%)	2 (5.7)	4 (3.8)	0.64
Atrial fibrillation, n (%)	0 (0)	1 (1)	1.000
Thyroid dysfunction, n (%)	3 (8.6)	5 (4.8)	0.412
PAD, n (%)	0 (0)	4 (3.8)	0.572
Hyperlipidemia, n (%)	27 (77.1)	80 (76.2)	0.908
Use of drugs, n (%)			
Aspirin	16 (45.7)	49 (46.7)	0.922
Calcium channel blockers	14 (40)	35 (33.3)	0.474
Beta blockers	12 (34.3)	35 (33.3)	0.918
Insulin	10 (28.6)	12 (11.4)	0.016
Oral antidiabetic	33 (94.3)	86 (81.9)	0.076
Anti-hyperlipidemic	16 (45.7)	49 (46.7)	0.922
Laboratory parameters			
White blood cell, ×10^3/μL	8.46 (6.7–11.5)	7.58 (6.9–9.8)	0.329
Hemoglobin, g/dL	15.1 (12.9–15.9)	14.8 (13.3–15.7)	0.985
RDW, fL	45.4 (42.7–49.7)	41.7 (39.7–44.5)	<0.001
CRP, mg/dL	6.09 (3.27–12.3)	6.23 (3–10)	0.195
TSH, mIU/L	1.65 (1.18–2.8)	1.8 (1.18–2.34)	0.883
Fasting insulin, mIU/L	26 (14.8–36.1)	20.8 (14–29.2)	0.424
HOMA-IR	9.19 (4.09–15.8)	6.99 (4.11–12.95)	0.412
HbA1c, %	8.1 (7.3–10.2)	7.3 (6.7–9.1)	0.074
Fasting glucose, mg/dL	145 (121–206)	133 (107–213)	0.349
BUN, mg/dL	34.6 (30.9–49)	32 (24–41)	0.013
Creatinine, mg/dL	0.89 (0.82–1.04)	0.76 (0.61–0.91)	0.001
Albumin, g/dL	4.62 (4.25–4.93)	4.53 (4.3–4.67)	0.199
Triglycerides, mg/dL	173 (105–213)	178 (136.4–237)	0.35
HDL, mg/dL	36 (34–39.1)	37.3 (32–41)	0.975
LDL, mg/dL	111.1±35.7	112.4±38.5	0.921
Total cholesterol, mg/dL	193.8 (178.6–219)	196 (161.2–221)	0.885
ECG parameters			
ECG rate, /s	80.1±15.8	80.6±10.9	0.899
QRS, ms	105 (103–120)	86 (80–93)	<0.001
Echocardiographic parameters			
EF, %	60 (60–65)	60 (60–65)	0.424
IVS, cm	1.3 (1.2–1.4)	1.3 (1.2–1.4)	0.206

Continue...

Table 1. Continuation.

Variables	fQRS(+) (n=35)	fQRS(−) (n=105)	p-value
LVEDD, mm	54 (50–56)	52 (48–55)	0.146
LVESD, mm	29 (28–38)	28 (28–30)	0.022
LA, mm	41 (40–43)	39 (36–42)	0.001
E wave	0.6 (0.5–0.8)	0.6 (0.5–0.7)	0.424
A wave	0.9 (0.7–0.9)	0.8 (0.7–0.9)	0.422
Lateral e	9 (8–10)	9 (7–11)	0.855
Septal e	8 (7–9)	8 (6–10)	0.676
Lateral a	13 (11–15)	12 (9–14)	0.096
Septal a	11 (10–12)	10 (8–11)	0.011
IVRT	110.9±14.1	107.2±15.2	0.238
DT	200 (166–220)	180 (150–200)	0.038
Epicardial fat thickness, mm	9.96±1.38	7.34±1.03	<0.001

BMI: body mass index; BP: blood pressure; CAD: coronary artery disease; CHF: congestive heart failure; PAD: peripheral artery disease; RDW: red-cell distribution width; CRP: C-reactive protein; TSH: thyroid-stimulating hormone; HOMA: homeostatic model assessment for insulin resistance; BUN: blood urea nitrogen; HDL: high-density cholesterol; LDL: low-density cholesterol; ECG: electrocardiography; EF: ejection fraction; IVS: interventricular septum; LVEDD: left ventricular end-diastolic diameter; LVESD: left-ventricular end-systolic diameter; LA: left atrium; IVRT: isovolumetric relaxation time; DT: deceleration time.

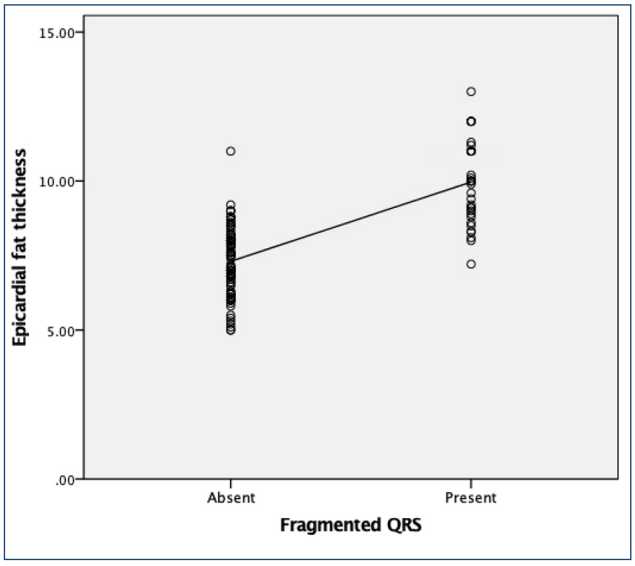

Figure 1. The correlation of epicardial fat thickness with fragmented QRS.

QRS duration were independent predictors of the presence of fQRS in MetS patients. These findings may support the thesis that at least some of the effects of MetS on cardiovascular diseases may be due to the accumulation of fat around the heart.

The EFT is in direct contact with the myocardium, and it is very metabolically active. The epicardial adipocytes can secrete a large number of cytokines and vasoactive peptides, including free fatty acids, interleukin-6, tumor necrosis factor-α, angiotensin II, and plasminogen activator inhibitor-1[15]. Recent studies have suggested that EFT with both local and systemic effects has an important role in deleterious effects of MetS on the heart[3,4]. Although the underlying mechanism is not clear exactly, EFT has also been shown to be associated with some ECG findings, such as AF, QT prolongation, left axis deviation, and low voltage in patients with MetS[6]. Obesity, hypertension, low-grade inflammation, oxidative stress, structural remodeling, and electrophysiological remodeling were all the proposed mechanisms[16]. However, the recent studies focus on myocardial fibrosis, which is the key histological component of cardiac remodeling in this context. Myocardial fibrosis, either in the form of interstitial, patchy, or dense scars, is shown to constitute a key histological substrate of arrhythmias[17]. Myocardial fibrosis is a well-recognized cause of morbidity and mortality[18]. Fibrotic scars of the cardiac muscle most commonly occur after myocardial infarction; however, there are various other conditions promoting myocardial fibrosis, such as hypertensive heart disease, diabetic hypertrophic cardiomyopathy, and idiopathic dilated cardiomyopathy[19].

The recent data have suggested that EFT, through its capacity to produce and secrete adipo-fibrokines (pro-fibrotic molecules) such as Activin A and MMP8, could be a complementary mechanism contributing to the formation of myocardial fibrosis[20]. Interestingly, a magnetic resonance imaging (MRI) study by NG has demonstrated that increased EFT volume index is independently associated with increased myocardial fat accumulation and interstitial myocardial fibrosis[21]. Myocardial fibrosis is a well-known underlying mechanism of fQRS occurrence in various cardiac pathologies[13]. Bekar et al. demonstrated fQRS as an independent predictor of EFT in hypertensive patients in their recent study[22]. Yaman et al. showed that the presence of fQRS was associated with increased EFT in healthy population[23]. In the light of these data, we

Table 2. Univariable and multivariable logistic regression analysis for detecting fragmented QRS.

Variables	Univariable OR (95%CI)	p-value	Multivariable OR (95%CI)	p-value
Gender, male	0.500 (0.230–1.085)	0.08	–	–
BMI	1.084 (0.999–1.177)	0.054	–	–
Waist circumference	1.057 (1.018–1.097)	0.004	0.987 (0.909–1.071)	0.748
Systolic BP	1.027 (0.996–1.059)	0.09	–	–
Diastolic BP	1.096 (0.987–1.216)	0.086	–	–
RDW	1.266 (1.127–1.422)	<0.001	1.033 (0.840–1.270)	0.758
HbA1c	1.129 (0.935–1.363)	0.207	–	–
BUN	1.008 (0.994–1.022)	0.270	–	–
Creatinine	2.672 (0.958–7.451)	0.060	–	–
QRS	1.344 (1.196–1.510)	<0.001	1.166 (1.076–1.264)	<0.001
LVESD	1.080 (1.001–1.164)	0.046	0.960 (0.813–1.134)	0.631
LA	1.159 (1.043–1.287)	0.006	0.947 (0.785–1.143)	0.573
Lateral a	1.136 (0.978–1.273)	0.103	–	–
Septal a	1.247 (1.049–1.483)	0.012	0.909 (0.657–1.256)	0.562
DT	1.008 (0.997–1.018)	0.152	–	–
Epicardial fat thickness	7.553 (3.484–16.373)	<0.001	3.441 (1.593–7.432)	0.002

BMI: body mass index; BP: blood pressure; RDW: red cell distribution width; BUN: blood urea nitrogen; LVESD: left ventricular end-systolic diameter; LA: left atrium; DT: deceleration time. Bold values indicate statistically significance (p<0.05).

speculate that increased epicardial fat content and its relation to myocardial fibrosis may explain the association of EFT with fQRS in subjects with MetS as demonstrated in the present study. Another interesting and important finding of our study is that it emphasizes the importance of the location of ectopic fat accumulation. WS is the major criteria of MetS and is used widely to assess visceral adiposity. In concordance with some relatively old trials that showed WS as a stronger cardiovascular risk predictor than body mass index (BMI)[24], the present study showed that fQRS(+) patients had higher WC and EFT than fQRS(–) patients, and both EFT and WC but not BMI were significantly associated with the presence of fQRS in subjects with MetS. These findings might suggest that information about ectopic fat distribution may provide important insights into metabolic and cardiovascular disease risk. Although WC lost its importance in multivariable logistic regression analysis, the small event size might contribute to this result and further studies with a large sample size might shed more light on this issue.

Limitations

Firstly, the small sample size was the major limitation. Secondly, we did measure EFT by echocardiography, but we did not measure any surrogate of myocardial fibrosis. We can only speculate about EFT's possible impacts on myocardial fibrosis and fQRS. In order to better understand the role of EFT in this process, MRI studies measuring both myocardial fat content and fibrosis are needed in subjects with MetS. The strength of the current study is that basic echocardiography and ECG may help risk stratification of newly diagnosed subjects with MetS by EFT and fQRS. Further studies with larger sample size could be more definitive about this issue.

CONCLUSIONS

The present study demonstrated the association of EFT with fQRS in subjects with newly diagnosed MetS. Basic echocardiography and ECG may help in risk stratification in subjects with newly diagnosed MetS.

AUTHORS' CONTRIBUTIONS

FŞ, TA, CŞ: Conceptualization. **FŞ, TA:** Data curation, Investigation. **FŞ:** Software, Validation, Formal Analysis, Writing – review & editing, Visualization. **FŞ, CŞ:** Methodology, Supervision, Writing – original draft.

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ORIGINAL ARTICLE

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Relationship between the number of prenatal care visits and the occurrence of adverse perinatal outcomes

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SUMMARY

OBJECTIVE: The objective of this study was to analyze the relationship between the number of prenatal care visits and the occurrence of adverse perinatal outcomes in an average city in the state of Minas Gerais, Brazil.

METHODS: This was a prospective and observational study. The variables analyzed were obtained through a questionnaire administered to postpartum women (between 1 and 48 h postpartum) and information contained in prenatal cards. The pregnant women were classified into three groups: Group I, <3 prenatal care visits; Group II, 3–5 prenatal care visits; and Group III, ≥6 prenatal care visits.

RESULTS: Group I had a lower median weight (57.0 vs. 64.0 kg, p<0.001), body mass index (22.1 vs. 24.3 kg/m^2, p<0.001), and weight gain (9.0 vs. 12 kg, p=0.002) than Group III. The prevalence of admission to the neonatal intensive care unit (11.6 vs. 4.2%, p=0.02) and the newborn mortality rate within the first 72 h of life (2.3 vs. 0%, p<0.001) were higher in Group I than in Group III. Group II had a higher prevalence of admission to the adult intensive care unit (5.7 vs. 0.6%, p<0.001) and a higher newborn mortality rate within the first 72 h of life (1.6% vs. 0%, p<0.001) than Group III.

CONCLUSIONS: Having ≥6 prenatal care visits was associated with lower rates of admission to the neonatal and adult intensive care unit, as well as a lower newborn mortality rate within the first 72 h of life.

KEYWORDS: Prenatal care. Obstetric delivery. Pregnancy outcome.

INTRODUCTION

Prenatal care includes a set of actions aimed at reducing the risk and severity of maternal and fetal morbidity and mortality[1]. The Brazilian Ministry of Health recommends at least six prenatal care visits: one, two, and three prenatal visits in the first, second, and third trimesters of pregnancy, respectively. In addition, these services must be indicated on the prenatal cards of pregnant woman, as this monitoring is used to diagnose and treat preexisting diseases or diseases that may arise during pregnancy[2].

Prenatal care can contribute to decrease adverse perinatal outcomes, especially in developing countries and socially disadvantaged populations[3]. A study conducted in a rural region of Rwanda reported that pregnant women who had only one prenatal care visit had higher rates of low birth weight and preterm delivery compared with those with ≥4 visits[4]. A study conducted in Ethiopia showed that ≤4 prenatal care visits was an independent predictor of adverse perinatal outcomes (i.e., stillbirths, fetal malformations, macrosomia, low Apgar scores)[5]. Likewise, among pregnant women from the traditional Bedouin community, lack of prenatal care was associated with adverse perinatal outcomes, and it was found to be an independent risk factor for preterm birth, low birth weight, and perinatal mortality[6].

In contrast, adequate prenatal care has been related to lower rates of obstetric complications, such as the hypertensive syndromes associated with pregnancy[7]. Prenatal supplementation with folic acid and iron reduced the risk of obstetric complications (e.g., postpartum bleeding, premature rupture of membranes, and puerperal sepsis) in pregnant women in a rural area of Nepal[8].

The objective of this study was to assess the association between the number of prenatal care visits and adverse perinatal outcomes in an average city in the state of Minas Gerais, Brazil.

METHODS

This was a prospective, observational, and descriptive study developed between August 2019 and July 2020 in the Department

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of Obstetrics and Gynecology of Mário Palmério University Hospital, Uberaba, MG, Brazil. The study was approved by the Research Ethics Committee of the University of Uberaba (UNIUBE) under CAAE no. 96383118.7.0000.5145. Consent forms were signed by all participants who voluntarily agreed to participate.

The inclusion criteria were women undergoing vaginal delivery or cesarean section during the study period. The exclusion criteria were women who refused to participate in the study and who were unable to complete the data collection instrument due to their inability to understand it.

The relationship between the number of prenatal care visits and the occurrence of adverse perinatal outcomes was analyzed in patients from the public, supplementary, and private health care systems. The number of prenatal care visits was assessed by analyzing the prenatal cards of pregnant women.

Serology performance during the prenatal period was considered adequate when the patient underwent the following tests at least once or repeated them for every 3 months: HIV, syphilis (VDRL), toxoplasmosis IgM and IgG, rubella IgM and IgG, cytomegalovirus (CMV) IgM and IgG, hepatitis B (HBsAg), and hepatitis C (anti-HCV).

Ultrasound evaluation during the prenatal period considered first-trimester ultrasound when performed between 6 and 13 weeks and 6 days, second-trimester ultrasound when performed between 20 and 24 weeks, and third-trimester ultrasound when performed between 28 and 40 weeks of pregnancy.

The evaluated variables were age; weight; smoking; alcoholism; use of illicit drugs; number of pregnancies; parity; type of health service; number of miscarriages; high-risk pregnancy; number of prenatal care visits; ultrasound examination in the first, second, and third trimesters; adequate serology (i.e., HIV, VDRL, toxoplasmosis, rubella, HBsAg, anti-HCV, and CMV); type of delivery (vaginal, nonelective cesarean section, elective cesarean section, or forceps); birth weight; 1-min Apgar score <7; admission to the neonatal intensive care unit (ICU); admission to the adult ICU; need for oxygen; neonatal hypotonia; intracranial hemorrhage; intestinal bleeding; neonatal infection; neonatal birth trauma; newborn death within the first 72 h of life; surgical site dehiscence/infection; maternal death; and composite perinatal outcomes.

The data were entered into an Excel 2010 spreadsheet (Microsoft Corp., Redmond, WA, USA) and analyzed using the SPSS version 20.0 and Prisma GraphPad software version 7.0 (SPSS Inc., Chicago, IL, USA). Quantitative variables were analyzed using the normality test (i.e., Kolmogorov–Smirnov) and presented as mean and standard deviation (SD) values. Variables with a non-normal distribution were presented as median, minimum, and maximum values. Categorical variables were described as absolute frequencies and percentages and represented in tables. The difference between categorical variables and their proportions was analyzed using the chi-square test. The Kruskal–Wallis test was used to assess the difference between continuous variables. Dunn's *post hoc* test was used to compare pairs. The significance level for all the tests was set at $p<0.05$.

RESULTS

During the study period, the data were obtained from 1,048 parturients. They were divided into three groups, as shown in Figure 1.

The following parameters had significant effects on the perinatal outcomes of the study group: age (p=0.010),

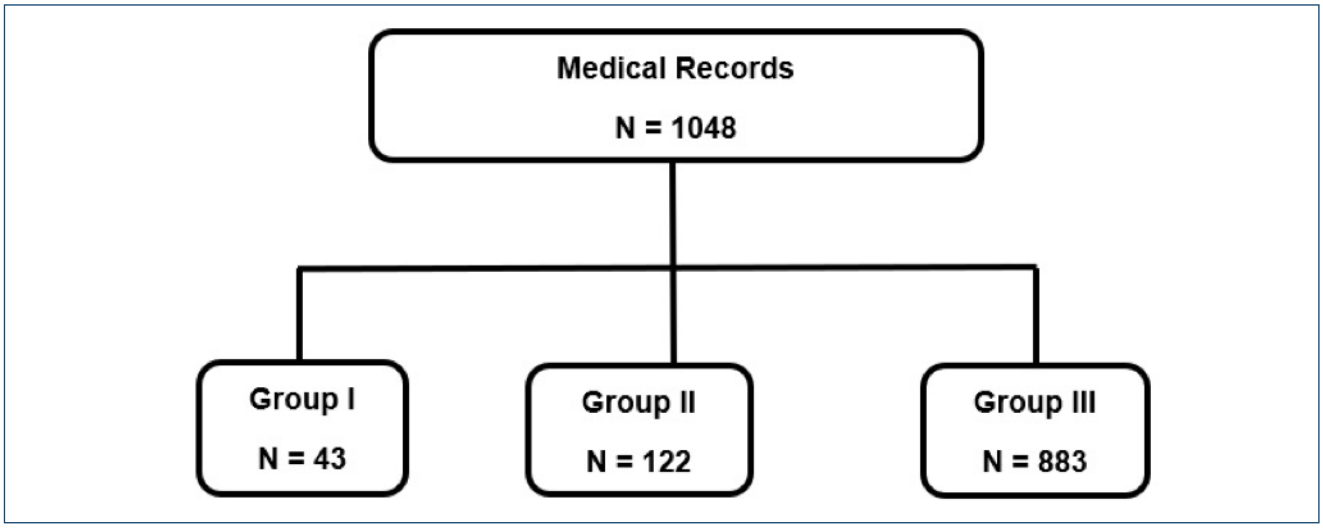

Figure 1. Flowchart of the cases included in the study.

weight (p<0.001), body mass index (BMI) (p<0.001), number of pregnancies (p<0.001), parity (p<0.001), weight gain (p=0.002), gestational age at delivery (p=0.015), and birth weight (p=0.003) (Table 1).

Comparison between pairs showed that Group I had a lower median maternal weight (57.0 vs. 64.0 kg, p<0.001), BMI (22.1 vs. 24.3 kg/m^2, p<0.001), and weight gain (9.0 vs. 12 kg, p=0.002) than Group III. Group II had a lower median maternal age (23.0 vs. 26.0 years, p=0.010), maternal weight (58.5 vs. 64.0 kg, p<0.001), BMI (22.7 vs. 24.3 kg/m^2, p<0.001), gestational age at delivery (38.9 vs. 39.0 weeks, p=0.015), and birth weight (3,078 vs. 3,210 g, p=0.003) than Group III (Table 1).

There was a significant association between the perinatal outcomes of the study group and smoking (p<0.001), alcoholism (p=0.003), illicit drug use (p<0.001), type of health service (p<0.001), first-trimester ultrasound (p<0.001), second-trimester ultrasound (p<0.001), third-trimester ultrasound (p<0.001), adequate serology, presence of a companion during labor (p<0.001), and type of delivery (p<0.001) (Table 1).

There was a significant association between the number of prenatal care visits of the study group and the prevalence of

Table 1. Clinical characteristics of the study population.

	Group I (n=43)	Group II (n=122)	Group III (n=883)	χ^2	p
Age (years)	25.0 (18–41)	23.0 (18–44)[c]	26.0 (18–48)	9.3	0.010[†]
Weight (kg)	57.0 (40–100)[b]	58.5 (40.2–130)[c]	64.0 (30–135)	16.8	<0.001[†]
Height (m)	1.60 (1.50–1.75)	1.60 (1.49–1.84)	1.62 (1.43–1.88)	2.2	0.328[†]
BMI (kg/m^2)	22.1 (16.4–39.1)[b]	22.7 (14.7–50.1)[c]	24.3 (12.3–47.8)	17.9	<0.001[†]
Smoking	34.9% (15/43)[a,b]	9.8% (12/122)	5.2% (46/883)	57.5	<0.001[ƒ]
Alcoholism	27.9% (12/43)[a,b]	13.9% (17/122)[c]	11.0% (97/883)	11.6	0.003[ƒ]
Illicit drug user	14.0% (6/43)[a,b]	2.5% (3/122)	0.6% (5/883)	57.1	<0.001[ƒ]
High-risk pregnancy	65.1% (28/43)	70.5% (86/122)	64.6% (570/883)	1.7	0.434
Number of pregnancies	3 (1–6)[b]	2 (1–10)	2 (1–13)	26.2	<0.001[†]
Parity	1 (0–5)[b]	1 (0–7)[c]	1 (0–9)	35.0	<0.001[†]
Type of health service				29.5	<0.001[ƒ]
Public	95.3%(41/43)[b]	90.2% (110/122)[c]	71.7% (663/883)		
Insurance	4.7% (2/43)[b]	9.0% (11/122)[c]	26.0% (230/883)		
Private	0% (0/43)	0.8% (1/122)	2.3% (20/883)		
Weight gain (kg)	9.0 (-11 to 27)[b]	10.0 (-23 to 51)[c]	12 (–12.5 to 34)	12.9	0.002[†]
First-trimester US	14.3% (6/42)[a,b]	30.6% (37/121)[c]	68.7% (594/865	108.0	<0.001[ƒ]
Second-trimester US	31.0% (13/42)[a,b]	74.4% (90/121)[c]	90.4% (782/865)	134.0	<0.001[ƒ]
Third-trimester US	71.4% (30/42)[b]	75.2% (91/121)[c]	93.3% (807/865	57.3	<0.001[ƒ]
Adequate serologies	42.9% (18/42)[a,b]	69.4% (84/121)[c]	89.9% (792/881)	101.0	<0.001[ƒ]
Gestational age at delivery (weeks)	39.0 (23.1–41.6)	38.9 (26.4–41.1)[c]	39.0 (32.1–41.7)	8.4	0.015[†]
Companion during the labor	86.0% (37/43)[b]	90.2% (110/122)[c]	97.3% (859/883)	25.6	<0.001[ƒ]
Type of delivery				35.1	<0.001[ƒ]
Vaginal	67.4% (29/43)[b]	63.1% (77/122)[c]	42.0% (371/883)		
Nonelective cesarean section	20.9% (9/43)	23.0% (28/122)	24.7% (218/883)		
Elective cesarean section	9.3% (4/43)[b]	13.1% (16/122)[c]	31.8% (281/883)		
Forceps	2.3% (1/43)	0.8% (1/122)	1.5% (13/883)		
Birth weight (g)	3,060 (305–3,765)[b]	3,078 (270–4,320)[c]	3,210 (1,260–4,970)	11.9	0.003[†]
1-min Apgar score	8 (2–9)	8 (1–10)	8 (1–10)	1.4	0.488[†]
5-min Apgar score	9 (2–10)	9 (1–10)	9 (4–10)	0.3	0.846[†]

BMI: body mass index; US: ultrasound.[ƒ]Chi-square: percentage (absolute number/total number). [†]Kruskal–Wallis: median (minimum–maximum).[a]Dunn's post-hoc test: group I vs. group II, p<0.05. [b]Dunn's post-hoc test: group I vs. group III, p<0.05. [c]Dunn's post-hoc test: group II vs. group III, p<0.05.

Table 2. Comparison of the frequency of perinatal adverse outcomes between the three analyzed groups.

	Group I (n=43)	Group II (n=122)	Group III (n=883)	χ^2	p
1-min Apgar score <7	14.6% (6/41)	9.8% (12/122)	6.7% (59/881)	4.84	0.089
Neonatal ICU admission	11.6% (5/43)[a]	8.2% (10/122)	4.2% (37/883)	7.87	0.02
Adult ICU admission	2.3% (1/43)	5.7% (7/122)[b]	0.6% (5/883)	23.8	<0.001
Oxygen need	27.9% (12/43)	26.2% (32/122)	26.0% (230/883	0.07	0.964
Neonatal hypotony	14.0% (6/43)	13.9% (17/122)	13.9% (123/883)	0.002	1
Intracranial hemorrhage	0% (0/43)	0.8% (1/122)	0.2% (2/883)	1.45	0.484
Intestinal bleeding	0% (0/43)	0% (0/122)	0.1% (1/883)	0.18	0.911
Neonatal infection	0% (0/43)	1.6% (2/122)	1.0% (9/883)	0.872	0.646
Neonatal birth trauma	0% (0/43)	0.8% (1/122)	1.6% (14/883)	1.1	0.578
Newborn death within the first 72 h	2.3% (1/43)[a]	1.6% (2/122)[b]	0% (0/883)	16.6	<0.001
Surgical site dehiscence/infection	0% (0/43)	0.8% (1/122)	1.7% (15/883)	2	0.536
Maternal death	0% (0/43)	0% (0/122)	0% (0/883)	*	*
Composite perinatal outcome	60.5% (26/43)	57.4% (70/122)	51.8% (457/883)	2.43	0.297

ICU: intensive care unit. Chi-square: percentage (absolute number/total number). [a]Dunn's post-hoc test: group I vs. group III, p<0.05. [b]Dunn's post-hoc test: group II vs. group III, p<0.05.

admission to the neonatal ICU (p=0.02) and the adult ICU (p<0.001), as well as the newborn mortality rate within the first 72 h of life (p<0.001) (Table 2).

The prevalence of admission to the neonatal ICU (11.6 vs. 4.2%, p=0.02) and the newborn mortality rate within the first 72 h of life (2.3 vs. 0%, p<0.001) were higher in Group I than in Group III. Group II had a higher prevalence of admission to the adult ICU (5.7 vs. 0.6%, p<0.001) and a higher mortality rate within the first 72 h of life (1.6% vs. 0%, p<0.001) than Group III (Table 2).

DISCUSSION

The provision of adequate prenatal care is an important public health concern worldwide. Inadequate prenatal care is associated with unfavorable perinatal outcomes[9]. In Brazil, factors related to the quality of prenatal care were associated with a high chance of death in premature newborns[10]. Places with scarce resources and without adequate and qualified obstetric care have a higher probability of stillbirths[11]. However, as demonstrated by Líbera et al.[12], for prenatal care assistance to effectively occur, it is crucial to involve pregnant women in issues related to their health.

Providing support for pregnant women and incorporating knowledge and skills through prenatal care can promote physical and mental health in minority women[13]. In this regard, the literature reports that geographic isolation, a major concern in Brazil, still affects women's health care and is associated with worrisome perinatal data[14].

In the present study, having ≥6 prenatal care visits was associated with more favorable perinatal outcomes, such as lower rates of neonatal and adult ICU admissions and newborn mortality within the first 72 h of life. These findings corroborate with the study by Santos et al.[15] that showed that having a minimum frequency of six prenatal care visits was a protective factor against low birth weight and prematurity.

The present study shows congruence with the new prenatal care model recommended by the World Health Organization (WHO), which raised the recommendation of the minimum number of prenatal care visits from 4 to 8. Some studies showed that an increased number of visits are associated with a lower probability of stillbirths. This fact is explained by the better opportunity to detect and manage potential adverse outcomes. This change decreases perinatal deaths by up to 8 per 1,000 live births when compared to the minimum of four prenatal care visits[16].

One of the limitations of the present study was that some of the data were collected from the prenatal cards of pregnant women, a document that always has a potential risk of adulteration, although this does not occur frequently. The relatively small sample size of the study patients may also have limited the accuracy of the results. The sample of the study patients may also represent a limiting element for obtaining even more

accurate data. The disparity between the public and private health systems in Brazil could also have been a hindering element in adequate data analysis.

Adequate prenatal care is influenced by complex elements that involve the acceptance of pregnancy and even the recognition of the need for specialized care at this important moment in life. To reach the minimum number of visits, there is need to overcome barriers such as lack of knowledge and the demand for population awareness programs. The training of health professionals and their engagement in prenatal care are considered important factors that can affect perinatal outcomes[17].

CONCLUSION

Having ≥6 prenatal care visits was associated with lower rates of neonatal and adult ICU admission and newborn mortality within the first 72 h of life.

AUTHORS' CONTRIBUTIONS

ABP: Conceptualization, Project administration, Supervision. **KMDR, CZ:** Data curation. **EFMS:** Formal Analysis, Writing – original draft. **CBOS:** Investigation. **EAJ:** Methodology, Writing – review & editing. **CZ:** Validation. **KMDR, CZ, EFMS, EAJ, CBOS, ABP:** Visualization.

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REVIEW ARTICLE

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Antifungal (oral and vaginal) therapy for recurrent vulvovaginal candidiasis: a systematic review and meta-analysis

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INTRODUCTION

Recurrent vulvovaginal candidiasis (RVVC) affects about 138 million women annually worldwide, with a global annual prevalence of 3,871 per 100,000 women[1]. Vulvovaginal candidiasis (VVC) is a common fungal infection caused by *Candida* species, predominantly *Candida albicans*. However, RVVC significantly compromises women's quality of life, causing severe symptoms of itching, pain, dyspareunia, dysuria, and leucorrhea. For this reason, the control of this recurrent infection remains a challenge for patients and experienced gynecologists[2-4]. RVVC is a condition arbitrarily defined as three episodes or more of VVC in the previous 12 months. However, some investigators demand yet another additional event, i.e., four attacks[2,3]. The etiopathogenesis of RVVC is still unclear. It is known that different elements are involved in this condition, such as immune mechanisms, genetic mutations, and behavioral patterns. However, the etiological factor remains unknown, hindering the clinical management of women with RVVC[5-7].

A significant number of topical and oral imidazole agents are available in various formulations with clinical and cure rates ranging from 80 to 90%[2-4]. Fluconazole has been the most used, and it is an inexpensive and well-tolerated antifungal drug that is easily administered orally. Meta-analyses realized about the theme demonstrate that fluconazole effectively reduces the recurrence of vaginal candidiasis up to 6 months after treatment[8,9]. However, in the last decade, fluconazole resistance has been reported in women with RVVC, consequence, in most cases, of the widespread availability of over-the-counter antifungal agent. Earlier epidemiological studies found that almost all women diagnosed with fluconazole-resistant *C. albicans* had experienced previous exposure to fluconazole[10]. While effective control of RVVC is achievable through using fluconazole maintenance suppressive therapy, the cure of RVVC remains elusive, especially in this era of fluconazole drug resistance. Ketoconazole and itraconazole are options of treatment found, as long as the cross-resistance is not determined[4].

Accordingly, our systematic review and meta-analysis aimed to assess antifungal treatment effectiveness for RVVC and provided an evidence-based protocol treatment for clinical use.

METHODS

This systematic review study with meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) guidelines[11]. The protocol of this systematic review is available in a previous publication[12].

Literature search and screening

PubMed, Embase, Scopus, Web of Science, SciELO, the Cochrane Central Registry of Controlled Trials (CENTRAL), CINAHL, and clinical trial databases, until July 2021, were used. Gray literature was searched using OpenGrey. No language restrictions were applied. The medical subject heading terms included: "candidosis," "vaginitis," "candida," "antifungal," "clotrimazole," "econazole," "butoconazole," "fenticonazole," "isoconazole," "miconazole," "omoconazole," "oxiconazole," "terconazole," "tioconazole," "sertaconazole," "natamycin," "amphotericin," "fluconazole," "ketoconazole," "itraconazole,"

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"posaconazole," "voriconazole," "nystatin" and were combined with Boolean "OR" and "AND" operators.

Eligibility criteria

Three researchers (JL, ACAS, and APFC) independently reviewed each article based on its title and abstract. The relevant data were collected by JL, RNC, and AKG. The inclusion criteria were as follows: randomized, blind, published clinical trials that analyzed women who had at least three episodes of vaginal candidiasis confirmed by the presence of signs and symptoms plus a positive vaginal culture for fungus, who had signs and symptoms plus positive vaginal microscopy compatible with vaginal candidiasis, and who had been treated with antifungal drugs administered intravaginally or orally. Studies with women immunosuppressive conditions or users of immunosuppressive drugs were excluded.

Data extraction

The clinical and mycological recurrence rate at 12 months, time to the first recurrence, and cure rate at 30 days were analyzed as the primary outcomes. The secondary outcomes were the proportion of participants with at least one recurrence during treatment and follow-up period, and complications/side effects.

A standardized data extraction form was used to collect the following data: authors, year of publication, country, the follow-up, mean age, the number of participants, interventions, and primary outcomes. The duplicate or secondary publications were excluded.

Quality evaluation

To assess the risk of bias, the Cochrane Collaboration bias risk tool was applied[13]. The studies were classified into "low risk of bias," "high risk of bias," or "unclear risk of bias." Two authors (JL and ACAS) assessed each original study and then qualified, and disagreements were resolved by consulting a third author (RNC).

Statistical analyses

The Review Manager software 5.3.3 was used to perform the meta-analysis. To evaluate the effectiveness of the proposed treatments, the dichotomous data were extracted from each study and inserted in a 2x2 contingency table. Then, we calculated the odds ratio (OR) for dichotomous data and mean weight difference (MD) for continuous data with a 95% confidence interval (95%CI) to obtain a global estimate summary. Heterogeneity was assessed by the I^2 statistic: (<25%, without heterogeneity; 25–50%, moderate heterogeneity; and >50%, strong heterogeneity). The fixed-effect model was chosen due to the low heterogeneity observed between studies. We used Egger's funnel plot to assess possible publication bias. A linear regression approach was used to assess the asymmetry of the funnel plot. Moreover, the outcomes that assessed the certainty of evidence were evaluated according to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool[14].

RESULTS

A total of 18,965 potential records were initially identified. Later, 118 additional records were identified. After review of the title and abstract, 78 full-text papers were reviewed, 13 studies met inclusion criteria, and 9 studies were included in the meta-analysis. A flowchart of the study selection process is shown in Figure 1.

This systematic review included 13 papers representing 1,552 women, with a mean age of 30.92 years. The study included seven studies from the United States, two from England, and one each from Sweden, Spain, Italy, and Iran. The general characteristics of all included studies were summarized and are shown in Table 1.

Four meta-analyses were performed as follows:

1. Mycological recurrence (seven studies)[15-17,20-22,26];
2. Second clinical recurrence (six studies)[15,17,20-22,26];
3. Average recurrence time (two studies)[26,27];
4. Effectiveness of clotrimazole with other antifungals (two studies)[23,24].

The meta-analysis for mycological recurrence at 12 months showed that the OR for people treated with fluconazole, ketoconazole, clotrimazole, and oteseconazole was 0.36 (95%CI: 0.24–0.55) when compared with untreated people. For clinical recurrence at 12 months, the OR for women treated with fluconazole, ketoconazole, and clotrimazole was of 0.36 (95%CI: 0.24–0.54) risk of clinical recurrence when compared with the control group. Meta-analysis showed that there is no difference of effectiveness when comparing clotrimazole with other drugs (fluconazole and ketoconazole) (OR: 0.76, 95%CI: 0.41–1.41). The women treated with fluconazole and itraconazole had an average recurrence time of 0.364 months (10.92 days) longer than untreated people. Presenting adverse effects were considered mild; for this reason, antifungal protocols were considered safe.

It was impossible to analyze subgroups between different classes of antifungals and topical and vaginal routes due to the diversity of outcomes, which would allow comparisons with a maximum of two studies each.

All studies were randomized; eight were double-blind, placebo-controlled trials[15,16,18,20,22,25-27]; only three trials described a good random sequence generation process and the methods used for allocation concealment[18,20,27]. The risk of bias for each included study is shown in Table 2.

According to the GRADE system, the studies provided strong and moderate evidence for all results. In general, the quality of evidence was strong due to the characteristics of the study design. The quality of evidence was downgraded one level because of the imprecision of the results (Table 1).

DISCUSSION

This study shows that clotrimazole, fluconazole, ketoconazole, and oteseconazole at different levels reduced the recurrence of VVC and decreased the fungal count in culture after 12 months

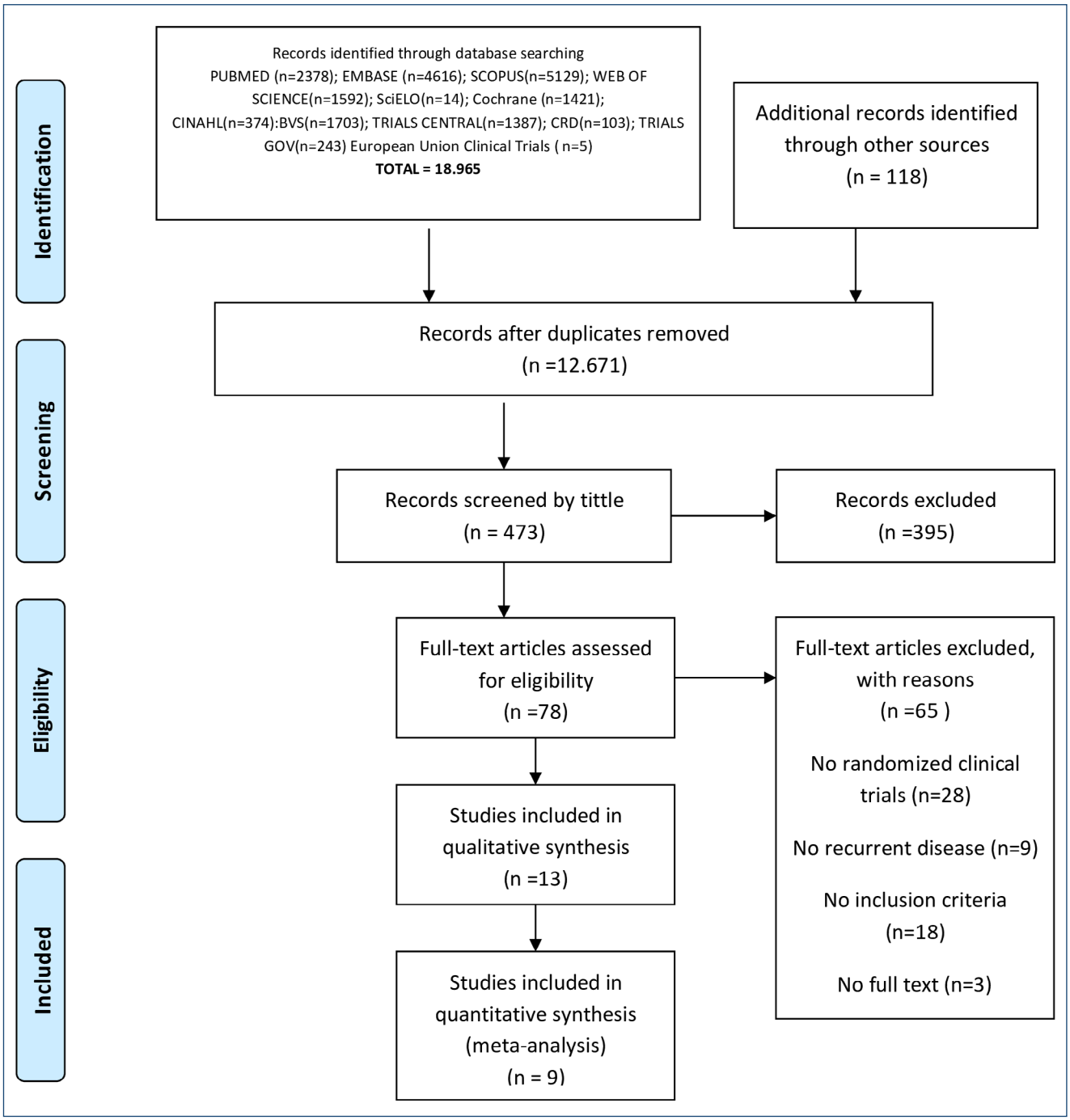

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram.

of treatment compared with placebo. Several studies evaluate the effectiveness of fluconazole in treating vaginal candidiasis; a minority refers to its use in treating CVVR. Donder's study evaluated the effectiveness and safety of an individualized, degressive, and prophylactic regimen in 136 women with RVVC. It was observed that individualized, degressive, and prophylactic maintenance therapy with oral fluconazole is an effective treatment regimen to prevent clinical relapses in women with RVVC[28]. The meta-analysis conducted by Rosa et al.[8] also suggests that fluconazole appeared to be the best drug. However, the latter highlights only the effectiveness of the drug in reducing symptoms. Two of the clinical trials included

Table 1. Characteristics of the studies included in the systematic review.

Author/year	Country	Interventions	N	Mean age	Follow-up	Outcomes	Certainty
Bolouri et al., 2009[15]	Iran	Weekly oral fluconazole´placebo	97	31.9 years (18–45)	12 months	Clinical recurrence and mycological recurrence	⊕⊕⊕⊕ HIGH
Brand et al., 2018[16]	EUA	Different dose-ranging of oteseconazole´placebo	215	34.6 years (18–64)	48 weeks	Proportion individuals with 1 or more episodes of CVV (culture) at week 48	⊕⊕⊕⊕ HIGH
Bushell et al., 1988[17]	England	Monthly clotrimazole vaginal tablet´placebo	41	27.8 years (18–41)	12 months	Clinical and mycological recurrence	⊕⊕OO LOW
Davidson et al., 1978[18]	England	Monthly vaginal clotrimazole´placebo	40	25.5 years (19–43)	10 months	Severity of symptoms during treatment; time to symptoms; time to reappearance of yeasts	⊕⊕⊕⊕ HIGH
López-Olmos et al., 2000[19]	Spain	Oral fluconazole´clotrimazole vaginal tablet´oral itraconazole	45	36.8 years (15-–53)	12 months	Clinical cure, mycological cure; recurrence	⊕⊕⊕O MODERATE
Roth et al., 1990[20]	Sweden	Monthly clotrimazole vaginal tablet´placebo	64	28.1 years	12 months	Clinical recurrence, mycological recurrence	⊕⊕⊕O MODERATE
Sobel et al., 1986[21]	EUA	Oral ketoconazole´placebo	63	32 years (19–47)	12 months	Clinical and mycological recurrence rate; time to clinical recurrence; adverse effects; disease-free patients	⊕⊕⊕⊕ HIGH
Sobel et al., 1989[22]	EUA	Monthly clotrimazole vaginal suppository´placebo	27	34.4 years (21–50)	12 months	Clinical and mycological recurrence rate; cure rate; mean time to symptom recurrence	⊕⊕⊕⊕ HIGH
Sobel et al., 1994[23]	EUA	Oral ketoconazole´vaginal clotrimazole	151	27.15 years (18–42)	2 months	Clinical cure and mycological cure; clinical recurrence; mycological recurrence; side effects	⊕⊕⊕O MODERATE
Sobel et al., 1995[24]	EUA	Oral fluconazole´clotrimazole vaginal tablet	432 (93 RVVC)	28.5 years (17–64)	35 days	Clinical cure and mycological cure; therapeutic response (clinical and mycological cure); recurrence rate; improvement of symptoms	⊕⊕⊕O MODERATE
Sobel et al., 2001[25]	EUA	Oral fluconazole one dose plus placebo´oral fluconazole 2 doses	556 (215 RVVC)	31 years (18–65)	35 days	Clinical cure and mycological cure	⊕⊕⊕⊕ HIGH
Sobel et al., 2004[26]	EUA	Weekly oral fluconazole´placebo	387	33.8 years (18–65)	12 months	Proportion of women in clinical remission at the end of the maintenance period (6 months) with definite cure	⊕⊕⊕O MODERATE
Spinillo et al., 1997[27]	Italy	Itraconazole vaginal tablet´placebo	114	30.5 years (18–50)	12 months	Clinical and mycological recurrence rate; proportion of patients free of symptomatic recurrence at 6 months and 12 months; mean time to symptom recurrence	⊕⊕⊕O MODERATE

Evaluated using the Grading of Recommendations Assessment, Development and Evaluation system: Very low ⊕OOO, Low ⊕⊕OO, Middle O⊕⊕⊕, High ⊕⊕⊕⊕[14].

in this review[15,25] did not demonstrate the effectiveness of fluconazole in clinical remission and the long-term mycological recurrence rate. A possible explanation for this ineffectiveness may be the presence of azole-resistant *Candida* species such as *Candida glabrata* and much less commonly *Candida krusei.*

The meta-analysis did not demonstrate the effectiveness of clotrimazole, itraconazole, and ketoconazole in the clinical remission of symptoms in women with RVVC. In their meta-analysis, Qin et al.[9] demonstrated the greater effectiveness of these drugs, including fluconazole. However, this study did not consider patients with RVVC, only patients with VVC. The difference of results can be justified because the randomized clinical trials (RCTs) that evaluated clotrimazole and ketoconazole included few patients, which may have influenced the absence of a significant difference, and we need to point the resistance azoles again.

An RCT with high-quality evidence, Brand et al.[16] showed that oteseconazole could be a promising new drug, decreasing the recurrence of symptoms and the reappearance of yeasts in the vagina. In addition, this new antifungal may be the most effective drug in *Candida* species resistant to other azoles[29]. The latter RCT was not included in the studies by Rosa et al.[8] and Qin et al.[9].

Regarding the proportion of participants with at least one recurrence during treatment and follow-up period, Sobel et al.[26] and Spinillo et al.[27] observed a higher rate of recurrences in the placebo groups. Fluconazole and itraconazole increased the time of occurrence of the first episode[26,27]. Clotrimazole, ketoconazole, itraconazole, and oteseconazole in the studies of moderate evidence are antifungal drugs with effectiveness for RVVC treatment. Fluconazole could reduce the rate of recurrence of symptomatic VCC. However, a long-term cure remains a challenge to achieve[16,22,23,26,27].

Table 2. Quality assessment of the included studies using the Cochrane risk of bias tool.

Study/year reference	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Bolouri et al., 2009[15]	?	?	?	?	?	+	?
Brand et al., 2018[16]	?	?	?	?	+	+	+
Bushell et al., 1988[17]	?	?	?	?	+	?	?
Davidson et al., 1978[18]	+	+	+	+	+	+	+
López-Olmos et al., 2000[19]	−	−	−	−	?	+	?
Roth et al., 1990[20]	?	+	+	+	+	?	+
Sobel et al., 1986[21]	?	?	?	?	+	+	?
Sobel et al., 1989[22]	?	?	?	?	+	?	?
Sobel et al., 1994[23]	+	?	?	?	+	+	+
Sobel et al., 1995[24]	?	?	?	+	+	+	?
Sobel et al., 2001[25]	?	?	?	?	+	+	+
Sobel et al., 2004[26]	?	?	?	+	+	?	?
Spinillo et al., 1997[27]	+	+	+	+	+	?	?

Key: − High risk of bias; ? unclear risk of bias; + low risk of bias[13].

The limitations of our study are based on potential missing data, biases, and heterogeneity in treatment protocols. However, this study included studies with new antifungals that professionals do not commonly use. Despite the immense diversity of treatment modalities, this study can illuminate potential targets for the treatment of RVVC, assuming that most of the randomized trials were evaluated with an unclear risk of bias.

CONCLUSIONS

This study provides moderate and high evidence that antifungal protocols using fluconazole, ketoconazole, and clotrimazole presented effectiveness for mycological and clinical recurrence rates when compared with placebo. The protocols using fluconazole, clotrimazole, ketoconazole, itraconazole, and oteseconazole were effective in the short-term treatment of RVVC. However, there was no difference in effectiveness between the drugs. In the long term, oteseconazole appears as a new effective drug compared with a placebo.

AUTHORS' CONTRIBUTIONS

JL, AKG: Conceptualization, Investigation. **JL, ACS, APFC:** Data curation, Writing – original draft, Formal analysis. **JL, PCG, AKG:** Methodology, Project administration. **JL, HS, RNC:** Software. **AKG:** Supervision. **RNC, HS:** Validation. **JL, ACS, AKG:** Visualization. **AKG, PCG, HS, RNC:** Writing – review & editing.

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REVIEW ARTICLE

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Acute thoracic aorta dissection: unraveling the pathophysiology of a silent killer

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INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality in the world and, among them, aortic diseases stand out, especially atherosclerosis, aortic aneurysms (AA), aortic dissections (AD), and aortitis. Acute aortic dissection (AAD) has a bad prognosis and presents itself by a sudden laceration of the intima and media layers of the aorta and its pathophysiology is correlated with atherosclerosis, aging, and inflammatory diseases such as vasculitis, in addition to connective tissue diseases, such as Marfan and Ehlers–Danlos syndromes[1]. This study aims to shed light on the pathophysiology and diseases associated with AAD, such an aggressive and deadly vascular disease.

ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS OF THE AORTA

The aorta is the largest artery in the human body, in caliber and length, with the largest number of tributary vessels and the largest volume of blood transported. It starts at the sinotubular junction and is divided into thoracic and abdominal aorta, based on the aortic hiatus of the diaphragm[1]. It is divided into three layers, namely, intima, media, and adventitia. The intima is made up of the endothelium and simple squamous epithelial tissue that lines the inner surface of the vessel. Between the intima and the media, there is an internal elastic lamina that makes up the subintima layer (Figure 1).

The middle layer is mainly composed of concentric layers of leiomyocytes, smooth muscle cells (SMCs), organized according to the vessel's length helically. They are responsible for the rigidity and elasticity of the aortic wall. Between the SMCs, there are variable amounts of elastic fibers and lamellae, collagen reticular fibers, adhesive proteins, proteoglycans, and glycoproteins. SMCs are responsible for the production of these extracellular matrix (ECM) molecules[2-4]. This layer has a thinner external elastic lamina that separates it from the adventitia. The amount of elastic laminae in the media layer plays an important role in energy conservation, regularization, and maintenance of blood flow at a constant value, preventing distension and loss of arterial anatomy. This elastic effect prevents the loss of kinetic energy in the blood, according to the determinations imposed by the Bernoulli equation, thus maintaining the total energy of the system.

Elastin and collagen are the main structural proteins of the middle layer, providing elasticity and tensile strength, respectively. Both proteins are stabilized by covalent cross-links and their formation is mediated by the enzyme lysyl oxidase (LOX). Cross-linking of tropoelastin monomers by LOX forms elastin molecules that cross-link with microfibrils to form elastic fibers and desmosines. In collagen cross-linking, pyridinoline is formed. Pyridinoline and desmosine stabilize, the cross-links of collagen and elastin, respectively[5,6] (Figure 2).

Finally, the tunica adventitia consists of loose connective tissue rich in collagen fibers, fibrocytes, and fibroblasts. This layer includes the *vasa vasorum*, responsible for the vascularization of the outermost layers of the aorta, in addition to the *nervi vasorum*, that promotes the contraction of SMCs through the noradrenergic action and lymphatic vessels for aortic self-regulation and metabolism[2-6].

THORACIC AORTIC DISSECTION

Diseases affecting the thoracic aorta can be categorized into acute or chronic. The most common are aneurysms, dissections, acute aortic syndrome (AAS), connective tissue diseases, and vasculitis. Among the causes of AAS, there is thoracic aortic dissection (TAD), which stands out for its high morbidity

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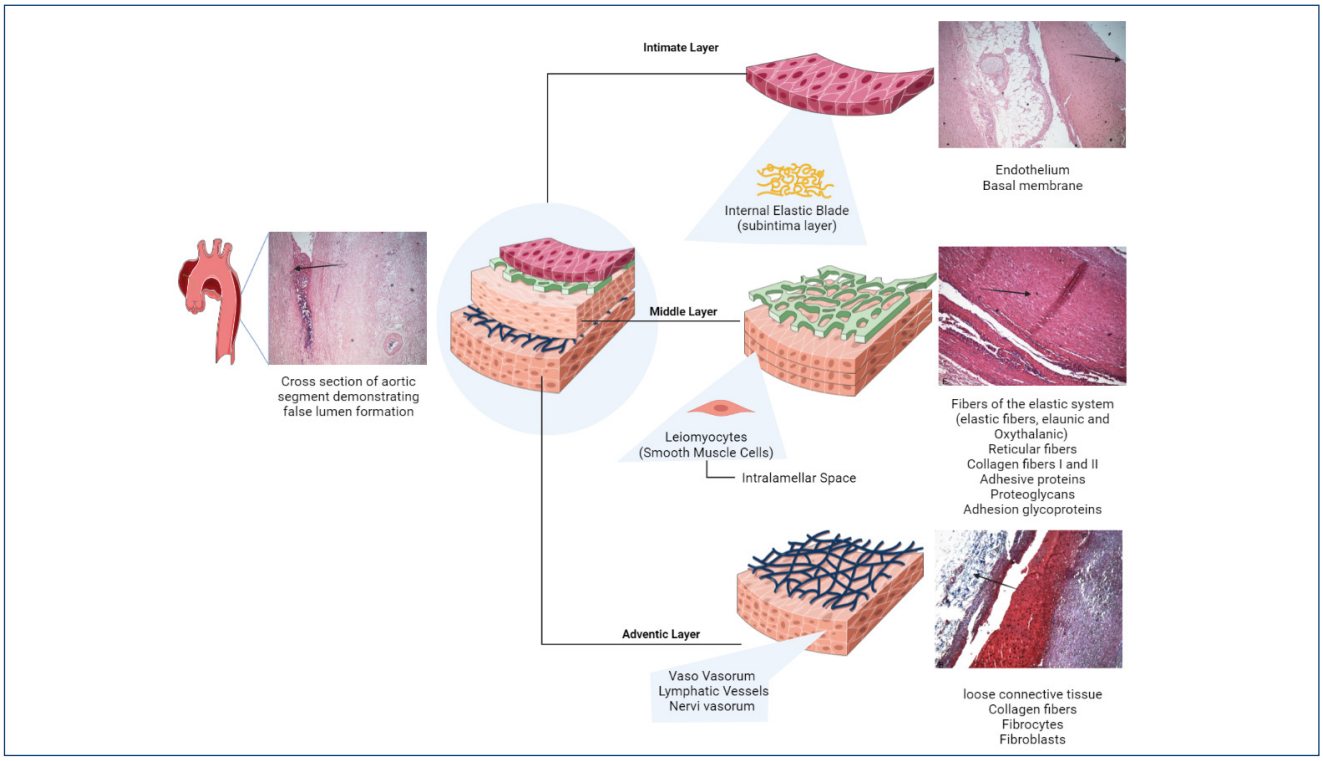

Figure 1. Schematic of a cross-section of aortic segment demonstrating the formation of the false lumen, the layers that make up the artery wall and its main structural components. Light microscopy images stained with hematoxylin and eosin were obtained from Professor Tenório et al. (2017)[5].

and mortality. Despite the differences in the pathophysiology of these diseases, systemic arterial hypertension (SAH) and atherosclerosis are the main participants involved in the pathogenesis of TAD, and SAH, in particular, is its greatest risk factor[7-9].

Thoracic aortic dissection begins with sudden lacerations and continuity breaks in the intima and media layers of the aorta. This rupture allows pulsatile blood to penetrate the medial layer and cause it to separate along the length of the vessel. This leads to the formation of a second channel, called the false lumen, leading to the relative thinning of the adventitial layer. Due to the mechanics of the injury, there is an accumulation of inflammatory cells throughout the aorta, which generates fragility of the architecture, dilation, and rupture. These changes can lead to disturbances in blood flow from the arteries tributary to the aorta, leading to multiple organ injuries secondary to TAD, and subsequently, high mortality[9-13].

THE IMPORTANCE OF ATHEROSCLEROSIS IN THE PATHOGENESIS OF THORACIC AORTIC DISSECTION

The accumulation of cholesterol and fat in the arteries accelerates collagen and elastin degradation, thus compromising the strength, structure, and elasticity of the aortic wall[5,6]. Atherosclerotic plaque formation is mediated by the immune system and significantly contributes to TAD. The first step in atherosclerosis is endothelial damage caused by sustained high

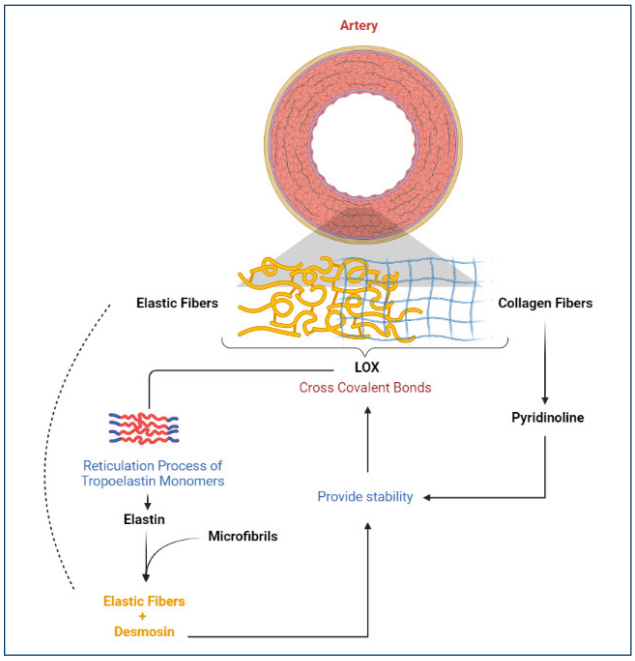

Figure 2. Middle tunic cross-linking process to provide elasticity and tensile strength. LOX: lysyl oxidase.

blood pressure, smoking, alcohol, and obesity. The endothelium is activated by the expression of adhesion molecules, in addition to high levels of interferon alpha and beta that are generated after activation of the Toll-like receptor 9. T cells produce pro-inflammatory mediators, such as interferon gamma (IFN-γ), which favors the adhesion of monocytes to the endothelium and their migration to the intimal layer, leading to a local inflammatory action[12-15].

Adherent monocytes to the intimal layer migrate to the subendothelial space in response to locally produced chemotactic molecules and differentiate into macrophages and foam cells. Macrophages are a rich source of growth-regulating molecules and cytokines that act as the main mediator of cell migration and proliferation. Due to this intense inflammatory reaction, there is activation of pro-apoptotic receptors in SMCs that are present after the subintima layer, so that there is a rupture of the interlaminar layer of elastin fibers. Apoptosis of SMCs and the consequent destruction of the ECM in the aortic wall are accompanied by an increase in the degree of inflammation. Several cytokines and chemokines that promote the recruitment of inflammatory cells to the aortic wall, such as tumor necrosis factor A (TNF-A), interferon gamma (IFN-γ), transforming growth factor-beta (TGF-β), metalloproteinases (MMPs)-1, MMP-9, and MMP-12, interleukin (IL)-1, IL-2, IL-6, and IL-8, can be found in the aortic wall. The presence of T lymphocytes, macrophages, mast cells, and neutrophils in the aortic wall evidences the stimulation of cell migration and its local harmful effect. This suggests that inflammation participates in the pathogenesis of TAD by regulating aortic wall homeostasis. T lymphocytes and macrophages can be found diffusely along the medial layer, in focal accumulations between the layers of SMCs, and within the wall of the vasa vasorum, suggesting its possible migration from the adventitia to the media of the aortic wall. These inflammatory cells are potential sources of proteases that can degrade the ECM and lead to aortic wall weakening, called tunica media degeneration[13-18].

Furthermore, TGF-β and MMPs promote aggregation of reactive oxygen species (ROS) or nitrogen intermediates, which induce apoptosis of SMCs and contribute to the degradation of interlaminar fibers. Recent studies have identified a number of molecular mechanisms that interact with the TGF-β pathway, including miR-29b, SMAD, activator protein-1 (AP-1), nicotinamide adenine phosphate oxidase-4 dinucleotide, and the mTOR pathway. They can lead to accelerated degradation of elastin and collagen fibers resulting in vascular wall weakness. In contrast, the increased expression and disordered collagen deposition may correspond to a slow repairing and pro-fibrotic process triggered by the fragmentation and depletion of elastic fibers. This fibrosis can also be due to an increase in the expression and release of growth factors that can culminate in an increase in collagen and consequent increase in arterial stiffness (Figure 3)[13-25].

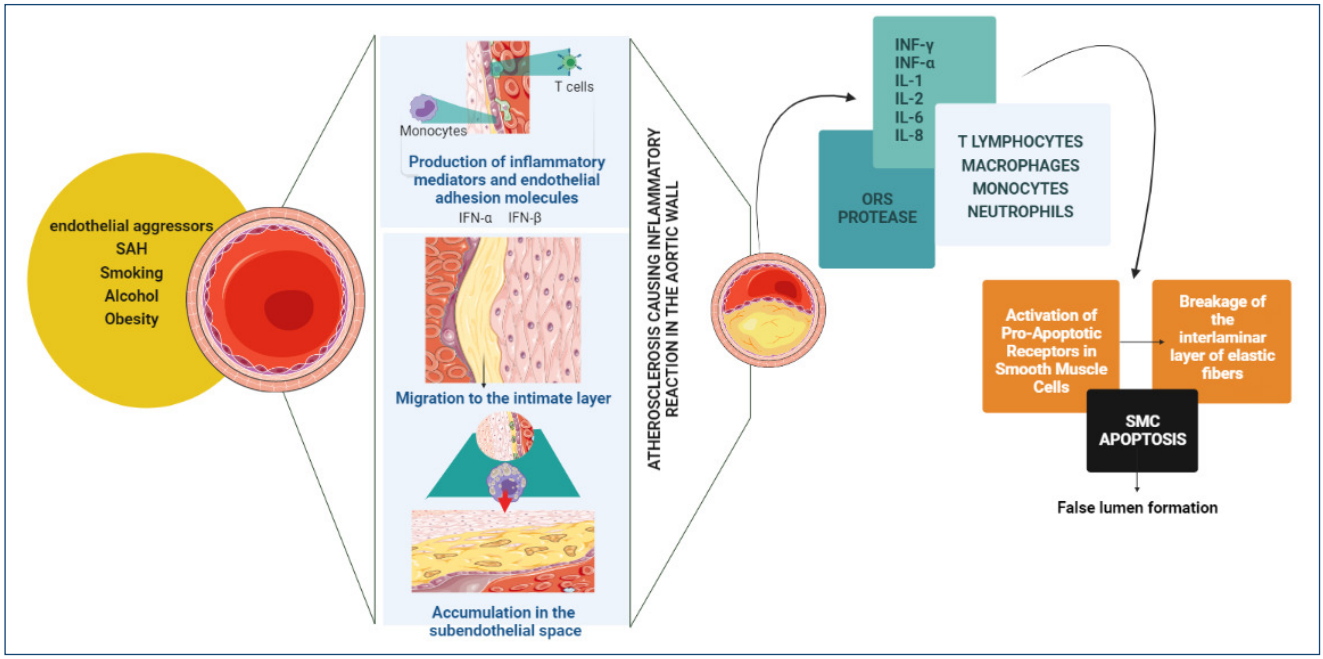

Figure 3. Atherosclerotic mechanism in the pathogenesis of thoracic aortic dissection. SAH: systemic arterial hypertension; IFN: interferon; IL: interleukin; ROS: reactive oxygen species; SMC: smooth muscle cell.

AORTIC DISEASES ASSOCIATED WITH THE DEVELOPMENT OF THORACIC AORTIC DISSECTION

Aneurysms, annuloaortic ectasia, aortic arch hypoplasia, coarctation, arteritis, vasculitis, bicuspid aortic valve, and genetic diseases that affect connective tissue, such as Turner, Marfan, Loeys–Dietz, and Ehlers–Danlos syndromes, are diseases involved in the development of TAD. These connective tissue disorders lead to aortopathies through overstimulation of TGF-β activity in the ascending aorta. However, only 20% of all TAD cases are due to syndromes. Consequently, non-syndromic TAD comprises the majority of cases and can be divided into familial, in which one or more family members have the disease, and non-familial. Interestingly, in cases of syndromic and non-syndromic TAD, often only one gene seems to be responsible for the pathophysiological mechanism of the disease. This creates the potential for early detection and preventive treatment, in contrast to diseases that have complex multigenetic and pathophysiological origins, such as atherosclerosis[18-26].

CONCLUSIONS

Thoracic aortic dissection is a clinical condition with high morbidity and mortality and is strongly related to aging, SAH, atherosclerosis, and connective tissue diseases. The absence of early predictors and screening methods are limiting factors in the treatment and control of damage caused by this disease. Therefore, it is urgent to carry out studies that aim at elucidating the pathophysiology and mechanisms, which are still obscure, that result in the development and validation of biomarkers.

AUTHORS' CONTRIBUTIONS

RM: Conceptualization, Methodology, Writing – review & editing. **CRN:** Conceptualization, Writing – review & editing. **JHAPF:** Writing – review & editing. **PPT:** Conceptualization, Supervision, Methodology, Writing – review & editing.

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COMMENTARY

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Comment on "Coronavirus disease 2019 pandemic and anxiety: a longitudinal study in 287 Brazilians"

Ran Hou[1], Tinghui Li[1]*

Dear Editor,

We are pleased to read the interesting paper published by Souza et al.[1]. Their findings revealed that a high prevalence of anxiety lowered after 8 weeks. Anxiety was worse in women, in youngsters, and in those who worked or studied in the area of health. Although this study is of great significance for the prevention of anxiety during the coronavirus disease 2019 (COVID-19) pandemic, I believe that some issues should be raised.

To begin with, the main problem of this article is the lack of describing the general demography in detail, such as gender, age, place of residence, economic level, educational level, occupation, and so on. Later, the results showed that women and young people were more easily to become anxious; however, most of the participants of this research were women and young people, which might cause selection bias.

In addition, the research only found that women and young people were more easily to become anxious, but further discussion on the influencing factors of anxiety among the women and young people should be described. Finding out the risk factors of anxiety among women and young people has an important guide value to the intervention of the COVID-19.

Finally, another concern is that the definition of anxiety and the range of age for young people should be provided in the methods part of this study. The maximum score of instruments was 63 points, and the participants were classified according to the degrees of anxiety: minimum: 0–10, mild: 11–19, moderate: 20–30, and severe: 31–63. Meanwhile, the cutoff value of defining anxiety should be offered in this study.

AUTHORS' CONTRIBUTIONS

RH: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **TL:** Conceptualization, Data curation, Formal Analysis.

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In the manuscript "Associations of high-mobility group box 1 and receptor for advanced glycation end products with acute lung injury in patient with acute aortic dissection", https://doi.org/10.1590/1806-9282.20210395, published in the Rev Assoc Med Bras. 2021;67(9):1251-1255, on page 1255:

Where it reads:

AUTHORS' CONTRIBUTIONS

JY: Conceptualization, Data curation, Formal analysis. **ZZ:** Funding acquisition, Investigation. **KZ:** Methodology, Project administration. **JC:** Supervision, Validation, Visualization. **HW:** Writing-original draft.

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JY: Conceptualization, Data curation, Formal analysis. **ZZ:** Funding acquisition, Investigation. **KZ:** Methodology, Project administration. **JC:** Supervision, Validation, Visualization. **HW:** Writing-original draft. **ZZ** and **KZ:** Contributed equally.