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Chemotherapy and chemoembolization of patients with oncopathology as a risk factor for the development of myocardial dysfunction

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Abstract

Considering the aging of the population, the combination of cardiovascular diseases with oncopathology is gaining more relevance. Liver cancer occupies the 6th place in the structure of the incidence of neoplasms and the 3rd place in mortality from all oncological diseases. One of the main methods of treating patients with liver cancer is chemotherapy and chemoembolization, which significantly affect the myocardium, developing cardiotoxicity. Myocardial damage is reflected in the development of heart failure, which subsequently is the main cause of death in cancer patients.

Key words: liver cancer, cardiotoxicity, chemoembolization, treatment, heart failure

Introduction

Oncological diseases occupy the second place among the causes of death not only in the world, but also in the Republic of Kazakhstan, giving way to cardiovascular diseases [1-3]. Currently, the probability of diagnosing cancer during a lifetime is 40% in men and 38% in women [4]. Recent advances in early diagnosis, accurate staging, and more effective therapy have resulted in improved survival rates for cancer patients. Over 25 years (the period 1991-2015), cancer mortality decreased by 26%. This means a reduction in cancer deaths by about 2.4 million over this period [4]. With increasing cancer survival, an increasing proportion of these patients are living with long-term adverse effects and complications of cancer. Chemotherapy - anthracyclines, trastuzumab, cyclophosphamide, 5-fluorouracil, angiogenesis drugs, thyroid kinases, etc. increase the risk of cardiovascular diseases, having a direct toxic effect on the myocardium [5,6].

The purpose of the review: to reveal the problem of myocardial dysfunction in patients with oncopathology receiving chemotherapy and chemoembolization.

Cardiovascular diseases (CVD) remain the main cause of death and disability in developed countries, accounting for 32% of total mortality [7]. Despite the measures taken for prevention and treatment, the overall cardiovascular morbidity (CVM) over the past years of the 21st century in the Republic of Kazakhstan (RK) has increased by 2.5 times, from 6775.6 per 100 thousand population in 2001 to 16982 ,9 in 2020 [8]. For the first time registered CVD in adults (18 years and older) over these years increased from 1841.3 (2001) to 4378.6 (2020) per 100 thousand population, including arterial hypertension (AH) from 614.0 to 2138, 9, coronary heart disease (CHD) from 321.5 to 604.2 and cerebrovascular disease (CVD) from 210.3 to 433.7, respectively. The growth of CVD, along with other factors, is to some extent due to the increase in life expectancy of the population. The vast majority of CVD occurs in the elderly and senile,

but they most often have comorbid diseases. Approximately 80% of older people have three or more diseases [9-11], which significantly increases mortality, so with two or more diseases it reaches 82% [12,13].

The above circumstances become particularly relevant when CVDs are combined with oncopathology [14-17]. Cancer diseases remain the main cause of mortality in patients of all age categories, and their combination with CVD increases the risk of morbidity and mortality [18,19]. As the population ages and life expectancy increases, the number of cancer cases increases [20]. In view of the observed increase in CVD over the past years of the 21st century by 2.5 times, including newly diagnosed major CVDs among adults: AH by 3.48 times, IHD by 1.88 times and cerebrovascular diseases (CVD) by 2.06 times [8]. At the same time, the average life expectancy of the population of the Republic of Kazakhstan in recent years has increased from 68.4 (2010) to 77.1 (2020) by 2.7 years.

Conducted by K.C. Stoltzfuet al. [18] an analysis of 7,529,481 cancer deaths showed that 5.24% were due to heart disease and the CVD mortality rate was 10.61/10,000 person-years, and the standardized rate was 2.24 (95% CI: 2.23–2, 25). The authors noted that the risk of CVD mortality increases with age and increases in cancer survivors over time. Here, it should be noted that the newly diagnosed oncological incidence in the Republic of Kazakhstan has decreased over the past 20 years from 195.9 (2001) per 100 thousand population to 94.2 (2020), and mortality has also significantly decreased from 134.4 to 75.0 respectively. However, advances in the development of new therapies have not only improved the survival of cancer patients, but also increased morbidity and mortality from treatment side effects, in particular due to cardiotoxicity [9,10]. Along with this, observed in practice quite frequent comorbidity of oncopathology with constantly growing CVD, contributed to the development of a new direction in medicine - cardio-oncology [14,17,21]. One of the key issues of cardio-oncology is the study of the effect of chemotherapy drugs on the myocardium.

Currently, chemotherapy remains one of the main methods of cancer treatment [22]. Undoubtedly, the use of new chemotherapy drugs has significantly improved cancer survival rates. However, against this background, the problem of cardiotoxicity, which can be caused by many types of antitumor drugs, becomes even more urgent [23,24]. Thus, the use of drugs such as anthracyclines and kinase inhibitors is associated with the development of chronic heart failure [25,26] and, accordingly, the death of patients [27]. Among the complications of cardiotoxicity during chemotherapy, dilated cardiomyopathy has the most unfavorable prognosis, associated with extremely high two-year mortality, reaching 60% [28,29]. Complications caused by chemotherapy cardiotoxicity negatively affect the quality of life and survival of patients, regardless of the prognosis associated with the underlying disease [30] and, according to leading ACC/AHA experts, the risk of premature mortality from cardiotoxic complications may be higher compared to the risk of death from the tumor process.

Today cardio-oncology is developing at a significant pace, working groups are being formed within such world communities as ACC, AHA, ESC, RCO, developing regulatory documents in this area. There is a sufficient number of studies of cardiotoxicity when using systemic and targeted chemotherapy. Hooning MJ et al. [31], who studied the 10-year risk of developing cardiovascular complications (CVC) in patients who survived after cancer, and later Chowetal [32] found an increase in the risk of cardiovascular death from 1.3 to 3.6 times and up to 18.5 times more complications such as hypertension, diabetes mellitus and dyslipidemia compared with patients of the same

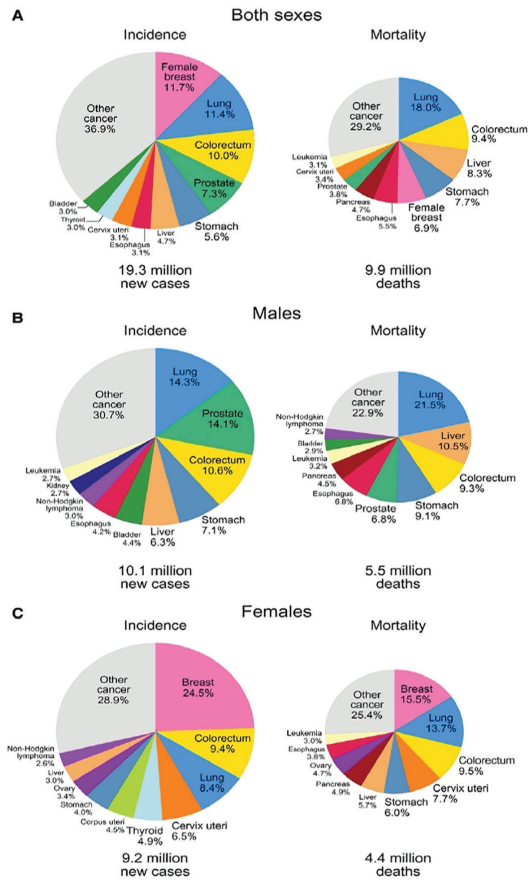

Figure 1 - Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries (https://acsjournals.onlinelibrary.wiley.com/doi/10.3322/caac.21660#:~:text=Worldwide%2C%20an%20estimated%2019.3%20million,cancer%2C%20with%20an%20estimated%202.3)

age group who do not have oncological pathology in history. The increased risk of CVD in cancer survivors is probably the result of an aging population combined with the direct and indirect [33] effects of cancer therapy, which extend to multiple systems [34]. According to Koelwyn et al. [35] CVD will become even more common among cancer patients as a result of the continued decline in cancer mortality combined with a rapidly aging population.

With the development of medical technologies, traditional surgical treatment of tumors is becoming less invasive [36]. The treatment of cancer patients is developing at a rapid pace. There is increasing use of minimally invasive techniques such as chemoembolization and radioembolization, used in hypervascular tumors by arterial embolization, such as hepatocellular carcinoma (HCC) [37].

HCC is the 3rd leading cause of death in cancer patients, accounting for more than 550,000 deaths worldwide each year with a 5-year survival rate at all stages of 8.6% [38-40]. The choice of treatment tactics depends on the degree of hepatic dysfunction and the general condition of the patient [41]. Transarterial hepatic artery chemoembolization (TACE) is successfully used in HCC, which is one of the common cancers with high mortality [42] and has become the gold standard of treatment in patients with HCC [43,44]. In traditional chemotherapy (intravenous and oral chemotherapy), the effectiveness of anticancer drugs is limited by the low concentration of the drug in the tumor, while nonspecific systemic toxicity of chemotherapeutic agents is observed, including cardiotoxicity and the development of drug resistance [45]. Delivery of a chemotherapeutic drug to the oncological focus in TACE, excluding systemic effects on

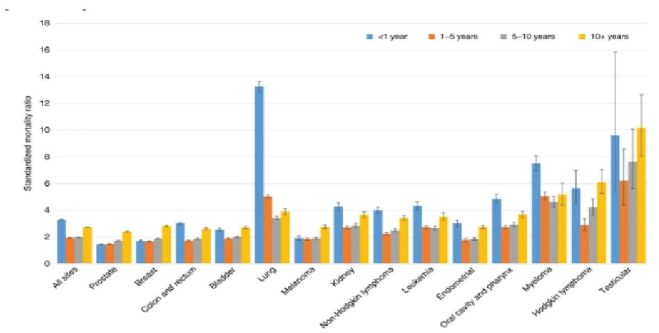

Figure 2 - Standardized mortality ratios (SMRs) of fatal heart disease among cancer patients by cancer subsite (https://www.nature.com/articles/s41467-020-15639-5 (with the author's permission)

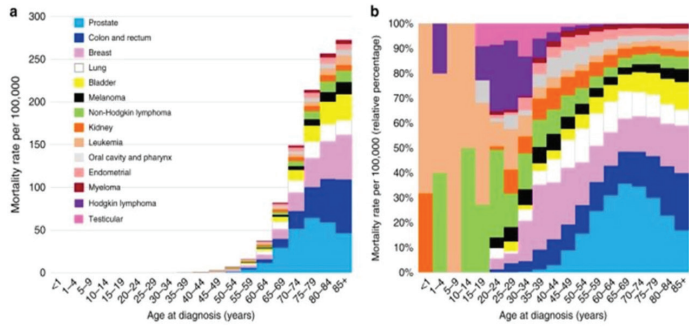

Figure 3 - Age adjusted mortality rates per 100,000 for fatal heart disease by cancer subsite. (https://www.nature.com/articles/s41467-020-15639-5 (with the author's permission))

the body, increases its specific effect on the tumor. Therefore, TACE is a widely used minimally invasive treatment technique that provides increased survival of patients with HCC [46,47]. However, the degree of influence of TACE on cardiotoxicity and, as a consequence, myocardial dysfunction has not been studied. All this has served as a reason to initiate a study on the influence of TACE on functional state of myocardium in patients with liver cancer. Patients with concomitant CVD and complications due to previous conventional chemotherapy are of particular interest in this regard.

Achieving positive results in cancer treatment is an important task for the healthcare system both in the world and in Kazakhstan. Despite the increase in scientific, clinical and biological knowledge about the prevention and treatment of malignant neoplasms, cancer remains one of the leading causes of enormous damage to the health care system [48]. However, behind the mask of malignant neoplasms, CVDs are often hidden, as well as well-known risk factors for their development and/or aggravation (smoking, sedentary lifestyle, heredity, age, etc.). Identified risk factors and early diagnosis of CVD can be corrected by prescribing preventive and therapeutic and rehabilitation measures. Thus, in the OUTCOME and PRADA studies, a positive effect of a prophylactic dose of angiotensin-converting enzyme inhibitors (ACE inhibitors) and b-blockers (BABs) on the myocardium was demonstrated. The primary outcome, the reduction in left ventricular ejection fraction from baseline to the end of the study, was -0.8% in the ACE-I group compared with the placebo group -2.6% (p = 0.026) [49]. It should be emphasized that the tactics of managing and treating cardiotoxicity in cancer are quite well and fully developed in traditional chemotherapy [13,14,17]. Modern innovative methods of administering chemotherapy drugs have not yet become widespread, and there are practically no studies on the management of patients with concomitant CVD and complications [50].

Conclusion

Modern cancer therapy has improved survival rates in patients with cancer, but the cardiotoxicity that develops with chemotherapy is a serious problem. To prevent morbidity and mortality from cardiotoxicity during anticancer therapy, especially in patients with cardiovascular disease, it is important to properly understand the underlying mechanism of myocardial dysfunction.

Questions about the effects of chemoembolization on the myocardium are still open. New studies in this direction will help to study in detail the clinical role of innovative therapies in the development of myocardial dysfunction.

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The role of intestinal translocation of E.coli in the development of acute obstructive pyelonephritis in an experiment

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Abstract

Aim: To study the role of *E. coli* intestinal translocation in the development of acute obstructive pyelonephritis in an experiment.

Material and methods: An experimental study was conducted on 60 male rabbits weighing 3000±500 g. The animals were divided into 3 groups of 20 animals each: experimental, control and intermediate control group. The acute obstructive pyelonephritis with the ureter blocking by laparotomy and introduction of the strain into the intestine were simulated in the animals of the experimental group. In the control group, the model was performed anologically as in the experimental group, but without the ureter blocking. In the intermediate control group, laparotomy was performed, the ureter was isolated without blocking and without the introduction of a bacterial strain. 10 animals of each group were removed from the experiment on the 3rd and 5th days, kidney tissue and urine were intake. As a reference marker strain, the laboratory strain *E. coli* No. 49579 was used, which was obtained from a patient with a urological infection and had resistance to cefepime, ciprofloxacin and tetracycline. Biomaterials were studied by microbiological examination and subspecific typing of strains using the MALDI-TOF MS method, antibiotic sensitivity was determined.

Results: *E. coli* strain was isolated in all animals of the experimental group and in 2 animals of the control group on the 5th day. During subspecific typing by the MALDI-TOF MS method, the isolated strains were identical in ribosomal proteins, and also had the same sensitivity to the said antibiotics. When analyzing the amount of lg CFU *E.coli* in urine after the experiment between the experimental and control group, we found that, on day 3, there were statistically significant differences between the groups (p=0.005), and on day 5, the amount of lg CFU *E.coli* was 13 times greater (p=0.004).A comparative analysis of the lg CFU *E.coli* index in kidney tissue on 3 (p=0.004) and 5 (p=0.003) days revealed statistically significant differences between the experimental group and the control group.

Conclusion: The results of identification and subspecific typing of isolated microorganisms confirmed that the strains isolated from the urinary tract were identical to the reference strain introduced into the gastrointestinal tract during the experiment, which confirms the role of translocation of intestinal microorganisms in the development of acute obstructive pyelonephritis.

Key words: urinary tract obstruction, acute pyelonephritis, intestinal translocation, *E. coli*

Introduction

Acute obstructive pyelonephritis is a common pathology among acute infectious and inflammatory diseases in urology and occupies about 14% in the renal diseases structure [1].

Infectious pathology occupies a leading position among kidney diseases and accounts for 11.1% of the total number of diseases of the urinary system. The main etiological factors are: *E. coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae* [2, 3].

Kidney disease occurs with a frequency of 766.8 cases per 100,000 people in the Republic of Kazakhstan. In 2020, 7625.7 cases of diseases of the genitourinary system per 100,000 people were registered in medical and preventive organizations [4].

R. D. Berg (1979) argued that one of the causes of bacteremia development is also bacterial translocation of microorganisms from the intestine. V. N. Titov and S. F. Dugina (2010) confirmed that microorganisms get into the blood after 15-20 minutes after introduction into the gastrointestinal tract (GIT) [5]. There are also experimental studies that confirm the intestinal translocation of bacteria into the blood and lymph nodes [6]. G. G. Gromova et al. (2019) showed that intestinal dysbiosis is the cause of infection of the urinary system. E. coli isolated from the urinary tract and intestines had the same set of biological properties [7].

About a hundred years ago, the phenomenon of bacterial translocation (BT) was first described as the process of microorganisms' penetration through the epithelial barrier of the intestinal mucosa into mesenteric lymph nodes, bloodstream and internal organs [8].

The increasing interest of scientists in the problem of BT is explained by the supposed possibility of penetrating bacteria and toxins to cause an inflammatory process in organs. Accordingly, it is very important to study not only the clinical and pathogenetic mechanisms of BT, but also microbiological and morphological, besides, research is needed in the field of diagnostics of the formation and development of diseases at BT.

Currently, several different models of acute pyelonephritis are known. Models of acute obstructive pyelonephritis are usually created by an obstruction in the ureter or urethra.

The methodology of «ascending» acute obstructive pyelonephritis modeling is described in detail in foreign literature. An infectious agent in the volume of 0.4 ml was injected into the bladder using an angiocatheter. In most cases, *E. coli* in the amount of 10^9 CFU/ml was used as a bacterial suspension. Then a clamp was placed on the urethra or the external opening of the urethra was closed for 4 hours, which ensured the reflux of infected urine into the kidney pelvis and the development of kidney inflammation. The authors described this technique as a model of bilateral reflux obstructive pyelonephritis [9,10].

P. V. Kosareva et al. (2008) proposed another model of acute pyelonephritis. After the kidney was isolated by laparotomy, a direct injection of an infectious agent (0.1 ml of *E. coli* culture in the amount of $5x10^9$ CFU/ml) was performed. According to the authors, this model corresponds to the clinic of acute non-obstructive pyelonephritis [11].

In recent years, the method of experimental modeling of acute pyelonephritis by open ligation of the ureter and subsequent injection of an infectious agent into the renal pelvis has been increasingly used. This technique was proposed by E. J. Giamarellos-Bourboulis et al. (2004). After premedication and anesthesia of the animal, the abdominal cavity was opened through an upper-median abdominal incision. After visualization, the ureter was surrounded by a thread distal to the pelvis and pulled up to the anterior abdominal wall. Both ends of the thread were passed through the anterior abdominal wall outward and tied on the skin, thereby causing partial obstruction. To create a complete obstruction of the ureter, a thread is wound under it and bandaged, followed by the injection of bacterial suspension into the renal pelvis at a concentration of 10^5 CFU/ml in 1 ml of saline solution through a needle [12].

Also, this technique was used in the work of M. I. Kogan et al. (2012). In the experimental work, the involvement of non-clostridial anaerobic bacteria in the etiology of acute obstructive pyelonephritis was studied. Animals (rabbits) were removed from the experiment on the 1st, 3rd, 7th, 14th and 21st days. The authors recorded foci of purulent inflammation and septic phlebitis in the wall of the pelvis and, to an even greater extent, in the cellular tissue of the renal sinus. It was also revealed that later purulent inflammation progressed, capturing the paranephrium and the system of collecting ducts, interstitial medulla [13].

Our study was devoted to the study of the role of *E. coli* intestinal translocation in the development of acute obstructive pyelonephritis. The pathogenesis of obstructive pyelonephritis is a topical issue in both clinical urology and urological research.

Material and methods

Before the experiment, each animal underwent general anesthesia by injecting ketamine into the femoral muscle area at a dose of 50 mg/ml in an amount of 1 ml once, calculated on a body weight of 15 mg/kg.

Animals of each group were removed from the experiment on 3rd and 5th day by exsanguination under general anesthesia, then material was taken for morphological and microbiological studies. The experiment involved 60 animals, which were divided into 3 groups of 20 individuals.

In the experimental group (n=20), acute obstructive pyelonephritis with blockage of the ureter was simulated to study translocation of *E. coli* from the intestine. In this group, an upper-median laparotomy 4 cm long was performed, a suspension of bacteria in the amount of 10^8 CFU/ml was injected into the small intestine 3 cm from Tracer ligament through a 26G needle. After visualization of the left ureter at the level of the upper third, the left ureter was tied with a 3/0 thread (Figure 1, 2).

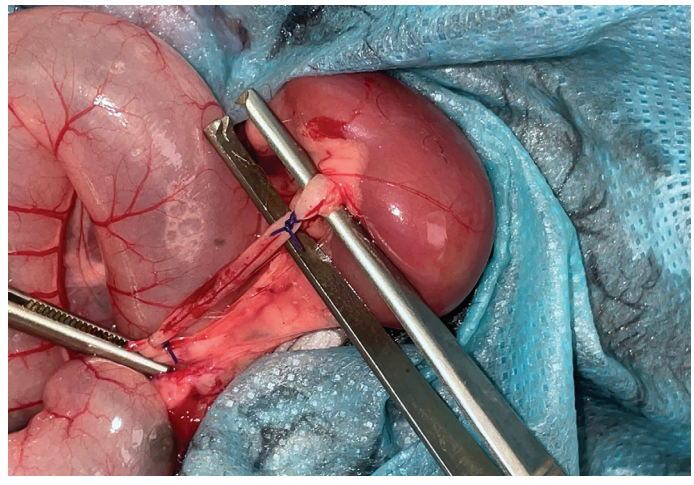

Figure 1 - Blocking of the upper third of the left ureter.

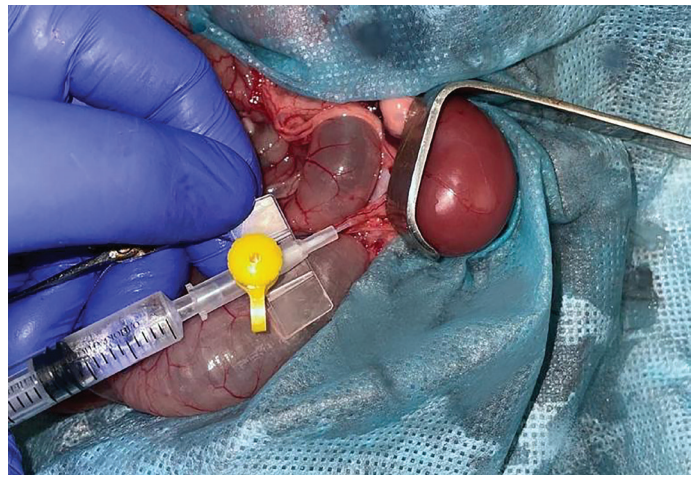

Figure 2 - Introduction of bacterial suspension into the ureter.

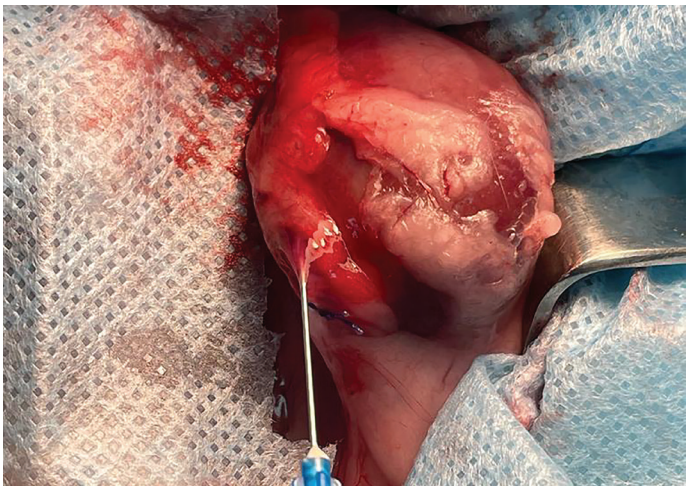

Figure 3 - Urine sampling from the renal pelvis.

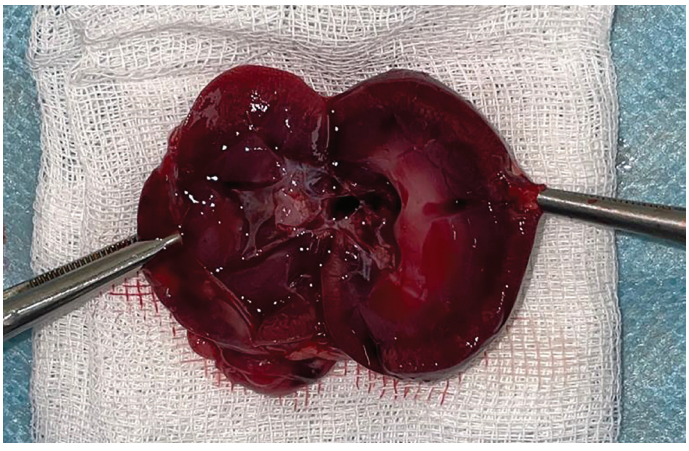

Figure 4 - Renal tissue sampling.

In the control group (n=20), animals underwent *Sham* surgery: an upper-median laparotomy 4 cm long, a suspension of bacteria in the amount of 10^8 CFU/ml was injected into the small intestine 3 cm from Tracer ligament through a 26G needle, the left ureter was isolated at the level of the upper third, but it was not bandaged.

In the intermediate control group (n=20), upper-median laparotomy was performed without ligation of the ureter and without injection of bacteria.

For microbiological examination, urine and kidney tissue above the level of obstruction were selected as the material (Figure 3, 4).

Biological material was taken from animals on the 3rd and 5th day. After taking the studied samples, the material in accordance with biosafety standards in a container was immediately delivered to the Shared laboratory of the Scientific-Research Center of NC JSC «Karaganda Medical University» [14], where primary plating on blood agar was carried out using a calibrated loop (10 µl) . Petri dishes with bacterial inoculation were incubated at a temperature of 37 °C for 24 hours. Plating for microflora was carried out on blood agar with 5% mutton blood with the release of pure cultures by conventional methods. Quantitative accounting of microorganisms in the studied material was carried out by counting the number of grown colonies on a Petri dish. The strains of microorganisms isolated during the study were considered clinically significant in the amount of >105 CFU/ml.

The strains that were in deep freeze were restored on nutrient agar (37 °C, 18 hours). Next, the samples were applied to the MBT Biotarget 96, dried in air with the application of 1 ml of saturated cyano-4-hydroxycinnamic acid (HCCA) matrix solution in 50% acetonitrile and 2.5% trifluoroacetic acid. The mass spectra were obtained using a Microflex LT Mass spectrometer (Bruker Daltonics) using default parameters (detection in linear positive mode, laser frequency – 60 Hz, ion source voltage – 2.0 and 1.8 kV, lens voltage – 6 kV) within m/z 2000-20000. 6 spectra were obtained for the strain in accordance with the Mass Spectrum Profile (MSP). External calibration of the mass spectra was performed using the Bruker Bacterial Test with ethanol/formic acid extraction in accordance with the manufacturer's recommendations (Bruker Colorblind, Bremen, Germany) [15].

The data files (obtained bacterial spectra) were transferred to flexAnalysis software (version 2.4; Bruker) for automatic peak extraction, as shown in Figure 5. Lists of peaks containing masses and intensities were exported as Excel files [16]. Sensitivity determination, as well as interpretation of the load to antimicrobial drugs – cefepime, ciprofloxacin and tetracycline were carried out by the disco-diffusion method on Muller-Hinton agar in accordance with EUCAST recommendations [17].

Statistical analysis

Statistical analysis was carried out using «Statistica 8.1 (Statsoft)» and StatTech v. 2.8.8. programs.

Quantitative indicators were evaluated for compliance with the normal distribution using the Kolmogorov – Smirnov criterion.

Quantitative data were described using median (Me) and lower and upper quartiles (Q1-Q3). The comparison of the two groups by a quantitative indicator, the distribution of which differed from the normal one, was performed using the Mann – Whitney U-test.

Ethics

The study design was approved by the decision of the Bioethics Committee of the NC JSC «Karaganda Medical University» (Protocol No. 7, dated 22.02.2022, assigned number No. 28).

Results

According to the results of the study, the following indicators of positive detection of *E. coli* in urine and kidney tissue were obtained, the occurrence frequency of which is indicated in Table 1.

In the experimental group, positive bacterial inoculation of *E. coli* was recorded – 100% positive results in urine and kidney tissue on the 3rd and 5th day. In the control group, where *Sham* surgery was performed without blocking the ureter with the injection of the strain into the intestine, on the 5th day, 2 animals were found to have positive E. coli culture, the number of CFU was 10^3, and it was not clinically significant. No bacteria were found in the intermediate control group (Table 1).

Table 1 Indicators of positive detection of E.coli bacteria in urine and kidney tissue

Group	day	N	% detection of bacteria in urine	% detection of bacteria in kidney tissue
Experienced group	3	10	100%	100%
	5	10	100%	100%
Control group	3	10	0%	0%
	5	10	20%	0%
Intermediate Control Group	3	10	0%	0%
	5	10	0%	0%

A comparative analysis of the values of *E. coli* lg CFU in urine after the experiment in all groups on the 3rd and 5th day is described in Table 2. From the presented data, it can be observed that in the experimental group, the level of E. coli lg CFU increased to 8 on the 5th day, which shows a statistically significant change (p=0,005). In the remaining groups, when comparing *E. coli* lg CFU index, no significant changes were detected depending on the modeling period (Table 2).

Table 2 Comparative analysis of lg CFU E.coli in urine on days 3 and 5 between the experimental and control groups

Group	day	lg CFU E.coli in urine after the experiment (CFU/ml)			Z	P
		Me	IQR	n		
Experienced group	3	8,00	7,00 – 8,00	10	2,835	0,005
Control group		0,00	0,00 – 0,00	10		
Experienced group	5	8,00	8,00 – 8,00	10	2,887	0,004
Control group		0,60	0,00 – 0,00	10		
Note: Me is the median, IQR is the interquartile interval, z -the value of the Mann-Whitney criterion, p -the significance level						

According to the presented table, when analyzing E. coli CFU amount in the urine after the experiment between the experimental and control groups, statistically significant differences (p=0,005) were found between the groups on the 3rd day. When conducting a comparative analysis of *E. coli* CFU amount in the urine after the experiment on the 3rd day, the strain was not recorded, there were no statistical differences between the control and intact rabbits. When comparing *E. coli* CFU values in urine after the experiment in all groups, a statistical difference was revealed between the groups on the 5th day. *E. coli* lg CFU in urine after the experiment in the experimental group was 13 times more than in the control group (p=0,004).

A comparative analysis of the *E. coli* lg CFU index in kidney tissue revealed statistically significant differences between the experimental and control groups (p=0,004). A comparative analysis of the values of *E. coli* lg CFU levels in kidney tissue on the 5th day in experimental groups is described in Table 3. It can be observed from the presented data that there was a significant difference between the experimental group and the control group (p=0,003) (Table 3).

Table 3 Comparative analysis of lg CFU E.coli in kidney tissue on days 3 and 5 between the experimental and control groups

Group	day	lg CFU of E.coli in kidney tissue after the experiment (CFU/ml)			Z	P
		Me	IQR	n		
Experienced group	3	8,00	8,00-8,00	10	2,887	0,004
Control group		0,00	0,00 – 0,00	10		
Experienced group	5	8,00	8,00-8,00	10	3,000	0,003
Control group		0,00	0,00 – 0,00	10		
Note: Me is the median, IQR is the interquartile interval, z -the value of the Mann-Whitney criterion, p -the significance level						

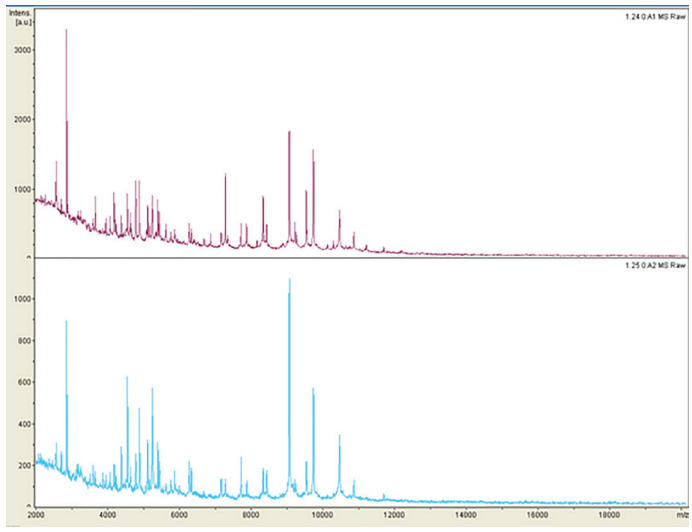

Figure 5 - Mass spectra of E.Coli isolated from urine from the category "isolates before the experiment/ isolates after the experiment".

As can be seen in Figure 5, when comparing 22 strains isolated from urine after acute obstructive pyelonephritis modeling, according to the sign «isolates before the experiment/ isolates after the experiment», 2 isolates were characterized by a coincidence of peaks. *E. co*li strains isolated from kidney tissue after the experiment were isolated with the same sensitivity profile to cefepime, ciprofloxacin, tetracycline.

Discussion

In the world literature, most researchers consider bacterial translocation as a pathological process that develops after extreme exposure and organ damage.

V. N. Titov et al. (2010) claim that the main cause of bacterial translocation is infection, stress, hypoxia, bacterial lipopolysaccharides, intestinal dysbiosis [18]. B. Ya. Usvyatsova et al. (2009) established that one of the causes of otitis is the translocation of bacteria to the focus of inflammation from the nasal cavity. The authors also claim that with an unfavorable course of otitis media, the pathogenicity of translocated strains increases [19]. In the work where the role of bacterial translocation in acute pancreatitis was studied, the authors revealed that translocated bacteria in patients with acute pancreatitis mainly consist of opportunistic microorganisms obtained from the intestine, including *E. coli, Shigella flexneri, Enterobacteriaceae, Acinetobacter lwoffii, Bacillus coagulans and Enterococcus faecium [*20]. Also, a number of authors claim that bacteria can pass the intestinal barrier in several clinical conditions, including excessive bacterial growth in the small intestine, impaired intestinal barrier and states of systemic immunosuppression. Bacterial translocation has also been detected in a wide range of other diseases, including depression, Alzheimer's disease, hemorrhagic shock, obstructive jaundice, abdominal surgery, malignant neoplasms, aortic aneurysm repair, heart failure, cardiopulmonary bypass and intestinal transplantation [21, 22].

Our study was devoted to comparing the role of ascending infection and intestinal translocation of *E. coli i*n the development of acute obstructive pyelonephritis. The pathogenesis of obstructive pyelonephritis is an urgent issue in both clinical urology and urological research.

The aim of our research was to study the role of intestinal translocation of *E. coli* in the development of acute obstructive pyelonephritis.

As a result of this study, a model of acute obstructive pyelonephritis in rabbits has been developed, which compares favorably with previously known models of this pathology. The experimental model of acute obstructive pyelonephritis with the urethra blocking proposed by us assumes technical execution of obstruction by ligation of the ureter in rabbits. When modeling acute obstructive pyelonephritis with blocking of the ureter by laparotomy, mortality in rabbits was not registered. In the work of R. Fukushima et al. (1994) it is said that the process of bacterial translocation is intense throughout the intestine. But A. V. Zhigailov (1996) asserts that with intragastric strains injection, the level of translocation through the mucous membrane of the stomach and small intestine is higher than in the large intestine [23].

The results of the microbiological study found that all animals of the experimental groups had positive bacterial culture, unlike the control groups. At the same time, a comparative analysis of the CFU level of the marker strain in the experimental groups showed no statistically significant differences and was higher than 105, which is of clinical significance for the development of pyelonephritis.

During the experiment, positive urine culture was detected in 2 animals of the control group on the 5th day, the CFU amount of bacteria was 10^3. But at the same time, bacteria were not found in the kidney tissue of these animals. There are confirmed hypotheses that translocation of bacteria occurs even in healthy individuals [24].

When comparing the identification and subspecific typing of isolated microorganisms with the reference strain, a 100% match of all isolates was obtained. When identifying isolated *E. coli,* it was revealed that these strains were resistant to cefepime, ciprofloxacin, tetracycline, as well as *E. coli* strain No. 49579, which was used as a reference strain.

Based on the results of our study, we can conclude that obstruction at the ureter level is a trigger for intestinal microorganisms' translocation into the urinary tract. The limitation of the study was that this study is a pilot. But this statement requires further research, both in the experiment and in the clinic.

Conclusion

The experimental results indicate that microbial translocation from the intestine to the urinary tract plays a significant role in the development of obstructive pyelonephritis. Urinary tract obstruction is a trigger for intestinal translocation of microorganisms into the urinary tract.

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A comparative study between HoLEP and bipolar TURP in the treatment of benign prostatic hyperplasia

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Abstract

Introduction: Benign Prostatic Hyperplasia (BPH) is one of the most frequent diseases in men. The laser treatment for BPH has challenged TURP due to advances in laser technology, a better understanding of tissue-laser interactions and rowing clinical experience.

Objective: To evaluate the safety and efficacy of HoLEP, comparing it to Bipolar TURP.

Material and methods: This was a prospective study to evaluate the outcomes in BPH patients undergoing surgery by HOLEP and Bipolar TURP done between January 2018 to December 2019. A total of 80 Patients were enrolled, 40 undergoing HoLEP and the other 40 Bipolar TURP for BPH. The procedures were performed by a single surgeon. All patients with symptomatic BPH and who were candidates for surgical treatment were included. Patients with previous prostate surgery, urethral surgery, history of prostate cancer or neurogenic bladder were excluded.

Results: Baseline parameters were almost similar between both the groups in terms of age, IPSS, QOL, Q max, PVR, and gland size. Operative time and resected gland weight were more in HoLEP arm ($p<0.001$). Catheter time and Hospital stay were significantly low in the HoLEP group ($p<0.0001$). Hemoglobin drop was not significant ($p=0.148$). IPSS at three months was similar in both groups ($p=0.608$). Qmax improved significantly in both groups, with 18.87 ml/s in TURP and 17.87 ml/s in HoLEP with a p-value of 0.261. PVR and QOL were similar between the two groups ($P=0.914$ and $P=0.781$).

Conclusion: Both Bipolar TURP and HoLEP were effective in relieving BOO. HoLEP has equal efficacy compared to conventional bipolar TURP, with decreased hospital stay and catheter indwelling time. The learning curve of HoLEP is steep; however, it can be overcome gradually.

Key words: benign prostatic hyperplasia (BPH), transurethral resection of prostate (TURP), holmium laser enucleation of prostate (HOLEP), international prostatic symptom score (IPSS), post void residue (PVR)

Introduction

Benign Prostatic Hyperplasia in males is a prevalent condition that has been associated with the physiological process of ageing. The prevalence among 70-year-old men is around 40%. Transurethral Resection of the Prostate (TURP) is the gold standard surgical procedure for BPH [1]. P. Gilling and M. Fraundorfer devised a method of holmium-laser resection of the prostate (HoLRP) in 1996, which was later modified to Holmium LASER Enucleation of the Prostate with the introduction of the Morcellator (HoLEP) [2]. Enucleation-transurethral resection of the prostate (e-TURP) is an evolution of the conventional TURP. As a true anatomical enucleation, it mimics open prostatectomy. The fence at the HoLEP is performed in the layer between the surgical capsule and adenomatous tissue.

In contrast to TURP, the prostate tissue is resected from center to periphery, and in this manner, the

vessels are opened again and again until the capsule level is reached. Because of developments in laser technology, a better knowledge of tissue-laser interactions, and significant clinical experience, the HoLEP laser therapy for BPH has challenged TURP. The holmium: YAG laser is a pulsed solid-state laser with significant advantages for endourological surgery. It has a wavelength of 2140nm, which permits it to be strongly absorbed by tissue water, resulting in fast vaporisation of exposed tissues at a depth of around 0.4mm and coagulation 3 to 4mm beyond the vaporisation surface. This results in a precise, bloodless field that prevents systemic fluid absorption [3]. HoLEP appears to be tough to learn and needs significant endoscopic expertise. The lengthy learning curve has hindered its popularity in the urological field [3].

This study is done to evaluate the safety and efficacy of Holmium Laser Enucleation of the Prostate (HoLEP), comparing it to Bipolar TURP.

Material and methods

This is a single-center, prospective, observational research to assess the results of BPH patients who underwent HoLEP or Bipolar TURP surgery between February 2018 and January 2019. The research included 80 patients receiving HoLEP and Bipolar TURP for BPH. All patients with symptomatic prostatic hyperplasia who were surgical candidates were included in the study. Patients having a history of prostate cancer, urethral surgery, or neurogenic bladder were excluded from the study. The local ethics committee authorized this study. After obtaining written informed permission, the patients were enrolled. Patients required to have subjective micturition complaints in the form of an AUA symptom score, maximal urine flow rate Q max in the uroflowmetry, and PVR to be evaluated in the research. To rule out any urinary tract infection, a comprehensive urine examination and urine culture were performed in the laboratory. If necessary, a significant urinary tract infection was treated preoperatively with antibiotic coverage. A serum PSA value exceeding 4 ng/ml and a striking digital rectal palpation were indications to carry out a TRUS guided 16 core biopsy when suspected. The patients who had a stricture of the urethra or prior prostate surgery, h/o urethral stricture, bladder tumor, or large bladder diverticula were excluded from the study. Perioperative factors assessed were age, international prostate symptom score (IPSS), peak flow rate (UFR), prostate volume and post-voiding residual urine (PVR). Intraoperative time, mucosal bladder injury, resected gland weight, any intercurrences were recorded. Postoperatively, hemoglobin drop, catheter indwelling time and hospital stay were noted. After 90 days, an assessment of IPSS, UFR and PVR was done.

Statistical analysis

MS Excel was used to enter data values, while IBM SPSS version 24.0 was used for statistical analysis. Data values for continuous variables were reported as mean and standard deviation. The Students t-test was performed to compare the mean differences between the two groups. All p-values less than 0.05 were deemed statistically significant.

Results

We enrolled 80 patients for the study, out of which 40 patients were in the HoLEP group and 40 patients in the TURP group.

Baseline parameters: In the study groups the mean age in TURP and HoLEP was 66.98 and 69.05 years respectively. Mean IPSS symptom score preoperatively was 25.85 in TURP group and 26.33 in the HoLEP group. Base line IPSS QOL was 5.05 in TURP group and 4.98 in HoLEP group. Base line Flow rate (Q max) was similar in both groups, 7.59 ml/s in in TURP and 7.15 ml/s in HOLEP group. Mean PVR in both the groups is comparable, with 195.75 ml in TURP and 197 ml in HoLEP group. Mean Prostatic volume measured pre-operatively by Ultrasound abdomen was higher in the HoLEP arm (52.1cc) compared to TURP (47.73cc) (Table 1).

Table 1 Comparision of base line parameters preoperatively in both study groups

	Base line Parameters	Group	N	Mean	Std. Deviation	p-value
1	Age (years)	TURP	40	66.98	9.47	.331
		HoLEP	40	69.05	9.50	
2	IPSS	TURP	40	25.85	4.817	.621
		HoLEP	40	26.33	3.668	
3	IPSS QOL	TURP	40	5.05	.504	.596
		HoLEP	40	4.98	.733	
4	Q max (ml/s)	TURP	30	7.59	2.22	.489
		HoLEP	33	7.15	2.73	
5	PVR (ml)	TURP	40	195.75	113.63	.966
		HoLEP	40	197.00	144.78	
6	Gland Size (grams)	TURP	40	47.73	15.66	.251
		HoLEP	40	52.10	18.06	

Mean operative time was higher in the HoLEP arm (66.5 mins) compared to TURP (52.03 mins). Resected gland weight was more in the HoLEP group (32.98 gms) compared to TURP (24.03 gms). Catheter indwelling time was comparatively less in the HoLEP arm vs TURP (3.17 vs 2.41 days). Total mean hospital stay was lesser in the HoLEP arm (3.13 days) compared to TURP (3.82 days). Hemoglobin loss was more in TURP than HoLEP (1.09 vs 0.91 gm/dl). Mean serum sodium change in TURP was 2.68 (meq/l) and in HoLEP it was 2.4 (meq/l).

The HoLEP arm had higher operating time and resected gland weight (p-value 0.001). The HoLEP group had considerably shorter catheter time and hospital stay (p-value 0.0001). The decline in hemoglobin was not significant (p=0.148), and the mean salt loss was equivalent to TURP (p=0.956) (Table 2, Figure 1).

Table 2 Comparision of operative and post operative parameters in both study groups

	Operative Parameters	Group	N	Mean	Std. Deviation	p-value
1	Operative time (minutes)	TURP	40	52.03	16.89	<0.0001
		HoLEP	40	66.50	18.33	
2	Resected Gland weight (grams)	TURP	40	24.03	11.84	.001
		HoLEP	40	32.98	11.27	
3	Catheter Time (Days)	TURP	36	3.17	.971	<0.0001
		HoLEP	37	2.41	.551	
4	Hospital Stay (Days)	TURP	40	3.82	.942	<0.0001
		HoLEP	40	3.13	.607	
5	Haemoglobin drop (gm/dl)	TURP	40	1.09	.65	.148
		HoLEP	40	.91	.44	
6	Sodium Change (meq/l)	TURP	40	2.68	1.61	.956
		HoLEP	40	2.70	2.38	

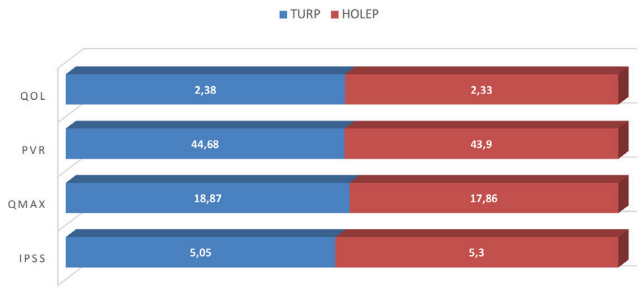

Figure 1 - Comparison of follow-up parameters at 3 months

Complications as graded by Clavien-dindo were comparable between the groups. We had 7 complications in TURP group of which 4 were Grade I, 2 were grade II and 1 was Grade III b complication. In the HoLEP arm we had 8 complications of which 5 were grade I, 1 was Grade II, and 2 were Grade III b complication. Clot retention with clots and stress leaks were considered Grade I, blood transfusion included in grade II, Re-surgery under anesthesia as Grade III b complication. Complications between the groups and was not statistically significant.

Follow up IPSS score at 3 months showed improvement in both groups (p-value = 0.608). Mean Q max was more in TURP than in HoLEP (18.87 vs 17.86 ml/s) with a p-value of 0.261. PVR post-operatively was similar in both the groups (p-value= 0.914). QOL improved in both the groups compared to pre-operatively (p-value=0.781) (Table 3, Figure 2).

Table 3 Comparision of follow up parameters at 3 months in Both study groups

	Follow up parameters	Group	N	Mean	Std. Deviation	p-value
1	IPSS	TURP	40	5.05	2.050	.608
		HoLEP	40	5.30	2.289	
2	Q max (ml/s)	TURP	40	18.87	3.65	.261
		HoLEP	40	17.86	4.34	
3	PVR (ml)	TURP	40	44.68	25.53	.914
		HoLEP	40	43.90	32.99	
4	QOL	TURP	40	2.38	.925	.781
		HoLEP	40	2.33	.656	

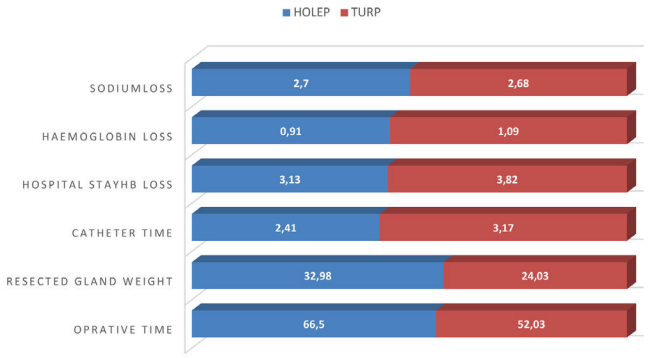

Figure 1 - Comparison of operative and post-operative parameters

Discussion

The present study is a clinical study of 80 cases, which were grouped into HoLEP and TURP, admitted in the urology department at our institute, from January 2018 to December 2019. Both the modality of surgeries was explained to patients, and the choice of surgery was decided by the patient. 40 cases were enrolled in each group and were analyzed and presented. In the study groups, baseline parameters like age, Q max, PVR, gland volume, IPSS and QOL were statistically comparable between both groups. In this study, we found symptomatic improvement of BOO in both groups; IPSS improved from 25.85 to 5.05 points in the TURP group and from 26.33 to 5.30 in the HoLEP group.

We observed the similar improvement in urine flow rate from 7.59 ml/s to 18.87 ml/s in the TURP group and from 7.15 ml/s to 17.86 ml/s in the HoLEP group at three months postoperatively. Operative duration and resected gland weight were longer in the HoLEP group than in the TURP group (p-value=0.0001 and p-value=0.001, respectively). Catheter indwelling duration (p-value=0.001) and hospital stay (p-value=0.024) were longer in the TURP group than in the HOLEP group. Hemoglobin drop and sodium loss in both groups were not significant (0.148 and 0.956). Complications, as graded according to the Clavien-Dindo system, were similar in both groups (p-value= 0.836). Follow up at three months with subjective IPSS and objective uroflowmetry was done. Q max, PVR were similar in both the groups at follow up with no significance obtained (p-value= 0.261, p=0.914).

At the follow-up, the IPSS score (p-value=0.717) and QOL (p-value=0.408) were not significantly different between the two groups. There is a considerable amount of literature on comparative comparisons of HoLEP and TURP, with multiple randomized trials indicating the perioperative benefits of HoLEP, the procedures superiority over TURP, with lower perioperative morbidity, improved effectiveness, and shorter catheter duration and hospital stay. At the 12-month follow-up, the results were comparable. Gilling et al. demonstrated that HoLEP is at least similar to TURP in the long run, with fewer re-operations required, in his longest, 7-year follow-up reported in 2011 on 61 patients, 31 in the HoLEP arm and 30 in the TURP arm [4]. Most of the studies have compared HoLEP with monopolar TURP. This makes a difference as the irrigations used in both the surgeries were different, normal saline in HoLEP whereas glycine in monopolar TURP. Studies on Bipolar TURP versus HoLEP, as in our study, are few. The irrigations used in both the surgeries were normal saline, and hence comparison between both the surgeries is more relevant. The baseline comparison of our study groups with other studies on bipolar TURP vs HoLEP is shown in (Table 4).

The sample size in our study was 40 cases of TURP and 40 of HoLEP. Studies in comparison are Fayad et al. [5] from Egypt did a prospective randomized study with a study group comprising of 30 patients each. Chen et al. [6] from china did a randomized study with a larger group comprising 140 patients each in both arms. Wilson et al. [7] from the UK did a retrospective study comparing TURP with HoLEP (425 vs 183). Imran mir et al. [8] from Pune, India, did a prospective study on 100 patients in each of Bipolar TURP and HoLEP arms. The mean age was 66.98 years in TURP vs 69.05years HoLEP in our study, whereas it is high in Chen et al. [6], Wilson et al. [7] study. Fayad et al. [5] study showed lower mean age than the present study (61.20 vs 60.06). Mean IPSS score was high in our study, compared to other studies. The mean Qmax was (7.59 vs 7.15), which was similar to all the study groups. Baseline gland size was comparatively smaller in our present study (47.73 grams vs 52.10 grams). In Fayad et al. [5] study, gland size was 80.60 vs 76.50, whereas, in Chen et al. [6], it was 60.31 vs 56.70, which was higher than the present study.

Various randomized studies on HoLEP demonstrated higher operative time than TURP and higher resected gland weight post-procedure. This is obvious as HoLEP involves

Table 4 Base line comparision of study groups with other studies on bipolar turp and holep (TURP versus HoLEP)

	Study	Year	Type of study	Sample size	Age	IPSS	Q max	PVR	Gland size
1	**Fayadet al[5]**	2011	Prospective Randomised	30 vs 30	61.20 vs 60.06	29 vs 27	6.98 vs 7.39	NA	80.60 vs 76.50
2	**Chen et al[6]**	2013	Prospective Randomised	140 vs 140	73.48 vs 72.11	23.27 vs 23.63	7.21vs 7.20	131.33 vs 128.16	60.31 vs 56.70
3	**Wilson et al[7]**	2013	Retrospective	425 vs 183	74 vs 72	NA	NA	NA	NA
4	**Imran mir et al[8]**	2017	Prospective observational	100 vs 100	NA	22.19	7.47	79.17	NA
5	**Present study**	2019	Prospective observational	40 vs 40	66.98 vs 69.05	25.85 vs 26.33	7.59 vs 7.15	195.75 vs 197	47.73 vs 52.10

removal of the larger gland, hence consumes more operative time and yields more resected weight. Our study also had a statistical significance in terms of higher operative time and higher resected gland weight in HoLEP. Comparatively, we had less operative time in our groups than other studies because of comparatively less mean baseline gland size in our groups. Operative time in our present study was 52.03 minutes vs 66.50 minutes in two groups.

HoLEP is advocated as having better hemostatic properties, and hence lesser irrigations and catheter indwelling catheter time are required, thereby decreasing hospital stay. Various randomized studies show a significant decrease in overall catheter indwelling time and reduced hospital stay favoring HoLEP. Catheter removal times were varied in multiple studies. We had a mean catheter removal at 3.17 vs 2.41 days in two groups. In other studies, Fayad et al. [5] catheter removal was at 24 hrs and 41 vs 48 hrs in Chen et al. [6] study. In Imran et al. [8], the mean catheter removal time was 2.52 vs 2.34 days. In the present study, we had a statistical significance in catheter indwelling time and hospital stays. Compared to other studies, we had a higher catheter indwelling time and hospital stay.

Studies are showing decreased haemoglobin loss in HoLEP, attributing to better LASER coagulation properties. Hemoglobin loss was significantly different in Chen et al., Wilson et al. study, Imran et al. study, Fayad et al. [5] study had no significant Haemoglobin loss. In our research, we had no considerable hemoglobin loss between the groups and the mean serum sodium change was also comparable between the groups.

Complications were graded in our study group based on modified Clavien-Dindo classification, and both the groups had similar complications and were not statistically significant [9]. Mamoulakis et al. [10] implemented the modified Clavien-Dindo classification system to standardize reporting complications in transurethral resection of the prostate in his study group of 198 patients reporting 44 complications and their grades. Also, Jong In Choi et al. [11] used the modified Clavien-Dindo classification for HoLEP surgeries and reported various grades in 402 patients. These studies have validated the classification system in endoscopic surgeries and found it an easily applicable tool for grading perioperative TURP complications.

Follow up in our study was done at three months with both subjective measures in terms of IPSS and objectively using urine flow rates. Both the groups demonstrated improvement in symptoms and urine flow rates compared to the baseline, and Q max and PVR was also not statistically significant between the two groups. Other studies, too, demonstrate the same results in terms of follow up.

In their evaluation using bipolar TURP, Gupta N et al. [12] shown that for big prostate adenoma (>60g), bipolar TURP and HoLEP gave equivalent results. HoLEP is a suitable endoscopic alternative to open prostatectomy for large glands and has proven its adaptability regardless of prostate size. The surgical therapy of a big prostate should be tailored to the patient's comorbidities and the surgeon's skills. Several research, including randomized and meta-analyses, have demonstrated that HoLEP is equal to TURP in terms of outcomes and effectiveness. The high learning curve of Holmium laser enucleation is its principal disadvantage [13-16]. The velocity of the operation can be used to evaluate a subjectively felt comfortable condition during the HoLEP treatment. Our findings revealed a gradual increase in efficiency.

The study's limitations include a non-randomized design, a short follow-up time, and a small sample size. Transurethral resection of the prostate (TURP) is the current standard surgical therapy for men with bothersome moderate-to-severe LUTS related to BPO, according to EAU 2018 recommendations on non-neurogenic male LUTS. When compared to TURP and open prostatectomy, Ho:YAG laser enucleation provides better haemostasis and intra-operative safety. With Level 1a evidence, peri-operative metrics such as catheterization time and hospital stay favour HoLEP, and the EAU highly recommends Ho:YAG laser enucleation of the prostate (HoLEP) to men with moderate-to-severe LUTS as an alternative to TURP or open prostatectomy [17].

Conclusion

Bipolar TURP and HoLEP were both beneficial in treating Bladder outlet obstruction. HoLEP has the same effectiveness as traditional bipolar TURP but requires less hospitalisation and catheter indwelling duration. However, as compared to the HoLEP surgery, bipolar excision of the prostate is a less expensive approach. Although the learning curve is high and there will never be a true plateau of knowledge, steady progress can be obtained as surgical volume increases.

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Evaluation of the relationship between vitamin D levels and emerge delirium in children who had tonsillectomy and/or adenoidectomy

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Abstract

Background: Pediatric emergence delirium is a general complication of anesthesia with an incidence of 2-80%. Although its etiology is not clearly known, it has been shown that anesthesia method, surgical procedure, and child and parent anxiety may cause the emergence delirium. The relationship between vitamin D levels and emerge delirium in children who underwent tonsillectomy and/or adenoidectomy has not been investigated before

Aim: In this context, this study was carried out to evaluate the relationship between vitamin D levels and emerge delirium in children who underwent tonsillectomy and/or adenoidectomy.

Material and methods: The study population consisted of children between the ages of 2 and 10, ASA I-II and were scheduled to have elective adenoidectomy and/or tonsillectomy under general anesthesia. All children (n=97) were evaluated for anxiety with the modified Yale Preoperative Anxiety Scale (m-YPAS) before surgery. Children with serum 25(OH)D levels<12 ng/ml and ≥12 ng/ml were categorized as group 1 (n=50) and group 2 (n=47). All children were evaluated for delirium with the Pediatric Anesthesia Emergence Delirium (PAED) scale. The face, legs, activity, cry, consolability (FLACC) scale was used in the evaluation of postoperative pain in children who could not express themselves verbally.

Results: The mean serum 1.25(OH)2D3 level was higher, albeit insignificantly, in group 1 than in group 2. There was no significant difference between the two groups in terms of emerge delirium, preoperative anxiety, postoperative pain and analgesia.

Conclusion: Vitamin D deficiency does not affect the incidence of emergence delirium in children. Vitamin D level does not relate to preoperative anxiety and postoperative pain.

Key words: child, delirium, adenoidectomy, vitamin D

Introduction

Pediatric emergence delirium is a general anesthesia complication with an incidence of 2-80%, in which behaviors such as shouting, crying, inability to make eye contact with parents, and agitation are observed during the recovery period [1]. Although its etiology is not clearly known, it has been shown that anesthesia method, surgical procedure, and child and parent anxiety may cause the emergence delirium [2]. In delirium, which usually occurs within the first 30 minutes of recovery, the patient may remove their catheter and injure him/herself and the site of surgery [2]. Hence, delirium causes an increase in the need for sedative and analgesic drugs, prolongation of the discharge time, and parental dissatisfaction [1].

Vitamin D deficiency is an important health problem in developed and developing countries. There are different threshold values used to define vitamin D deficiency in children [3]. High vitamin D levels are considered to protect cognitive functions, thereby preventing delirium [3]. Active vitamin D ($1.25(OH)_2D_3$) regulates intracellular calcium (Ca) balance and expresses neurotrophic factors necessary for nerve cell function. It is known that acetylcholine deficiency causes delirium, and the enzyme which synthesizes acetylcholine is regulated by vitamin D [3]. In order to determine the vitamin D level in the body, both 25(OH)D and $1,25(OH)_2D_3$ can be measured from the serum. Serum 25(OH)D has a longer half-life and its level is 1000 times higher than serum $1,25(OH)_2D_3$. Therefore, 25(OH)D measurement gives more accurate results in determining the vitamin D level. There are studies showing that vitamin D deficiency is associated with the development of emerge delirium in adult patients [4-6], however, a thorough review of the literature did not reveal any study that addressed emergence delirium in the pediatric patient population. In this context, this study was carried out to evaluate the relationship between vitamin D levels and emerge delirium in children who underwent tonsillectomy and/or adenoidectomy.

Material and methods

Study design and setting

This prospective, double-blind study was carried out between February 2019 and February 2020 (NCT05076162). The study protocol was approved by the ethics committee of Aydın Adnan Menderes University **(Approval No: 2019/11).** The study population consisted of children between the ages of 2 and 10, whose health statuses were classified as I or II according to American Society of Anesthesiologists (ASA) classification and were scheduled to have elective adenoidectomy and/or tonsillectomy under general anesthesia. Informed, verbal, and written consent was obtained from the legal guardians of all patients.

Children with previous anesthesia and/or surgery history, kidney-heart and liver failure, whose health statuses were classified as ASA III or IV, who had a neuromuscular disease, craniofacial anomaly, and rickets, and who have been using vitamin D and antipsychotic drugs were not included in the study.

ASA (American Society of Anesthesiologists) CLASSIFICATION

ASA 1. A healthy person with no disease or systemic problem other than normal, surgical pathology that does not cause a systemic disorder.

ASA 2. Person with a mild systemic disorder due to a cause requiring surgery or another disease.

ASA 3. Person with a disease that limits his or her activity but is not debilitating.

ASA 4. A person who has a disease that causes him to lose his strength completely and poses a permanent threat to his life.

ASA 5. A dying person who is not expected to live more than 24 hours, with or without surgery, and for whom surgery is the last hope.

ASA 6. It includes patients with advanced brain death.

Study procedures

The demographic data (age, weight, height, and comorbidities) of all cases were recorded within the scope of the preoperative evaluation. Children were not premedicated. All children were evaluated for anxiety with the modified Yale Preoperative Anxiety Scale (m-YPAS) before surgery [7,8]. The m-YPAS consists of five subscales: activity, vocalizations, emotional expressivity, state of apparent arousal, and use of parents. The scale consists of a total of 22 items. Six of these items are in the vocalizations subscale, whereas all other four subscales include four items each. The total scale score is calculated using the following formula: [(activity subscale score/4)+(emotional expressivity subscale score/4)+(state of apparent arousal subscale score/4)+(use of parents subscale score/4)+(vocalizations subscale score/6) x 100/total number of categories] [8].

Standard monitoring procedures, including the monitoring of heart rate, oxygen saturation, and non-invasive blood pressure, were implemented for all patients who were taken to the operating room for the surgical procedure. In all patients, anesthesia induction was achieved with 6 L/min flow and 100% oxygen and 8% sevoflurane concentration using appropriate face masks. When the adequate depth of anesthesia was achieved, peripheral venous cannulation was performed.

Blood samples were taken to measure serum 25(OH)D, $1.25(OH)_2D_3$, and calcium levels. Serum 25(OH)D levels were measured with the human enzyme-linked immunosorbent assay (ELISA) kit (catalog no: E1546Hu, #1008 Junjiang Inter. Bldg 228 Ningguo Rd, Yangpu Dist Shanghai 200090, China). The study groups were determined according to serum 25(OH)D levels. Accordingly, children with serum 25(OH)D levels<12 ng/ml and ≥12 ng/ml were categorized as group 1 and group 2, respectively [9].

Following the anesthesia, 1 mg/kg lidocaine, 1 μg/kg fentanyl, and 0.6 mg/kg rocuronium were administered intravenously. After 60-90 seconds had elapsed, endotracheal intubation was performed. Anesthesia was maintained with 3 L/min flow and 50% N_2O-O_2 and 2-3% sevoflurane concentration. In order to provide postoperative analgesia, 10 mg/kg of paracetamol was administered to all children intravenously. Details of the surgical procedure were recorded in the anesthesia follow-up form. At the end of the operation, 3 mg/kg sugammadex was administered intravenously for neuromuscular agent antagonism. After observing that spontaneous breathing was sufficient and protective reflexes returned, extubation was performed. Patients with spontaneous eye-opening and extremity movement were taken to the recovery room.

All children included in the study were evaluated for delirium with the Pediatric Anesthesia Emergence Delirium (PAED) scale at 10-min intervals from the time they were taken to the recovery room. PAED scale consists of five criteria. Accordingly, it assesses the child's eye contact with his/her caregiver, whether his/her actions are purposeful, whether he/she is aware of his/her surroundings, whether he/she is restless, and whether he/she is inconsolable. There are five choices to choose from in each criterion: “not at all”, “just a little”, “quite a bit”, “very much”, and “extremely”, which are awarded between 4 and 0 points in the descending order in the case of first three criteria and the ascending order in the case of the last two criteria. A PAED score of 10 or more is considered to indicate pediatric emergence delirium [10].

The face, legs, activity, cry, consolability (FLACC) scale was used in the evaluation of postoperative pain in children who could not express themselves verbally [11]. FLACC scale consists of five categories of face, legs, activity, cry, and consolability, each of which is assigned a score between 0-2 points. Hence, the overall FLACC scale score ranges between 0-10 points. Each category is assessed in 10-minute periods, totaling 50 minutes for the overall scale [12]. Patients who obtained a total score of ≥ 4 from the postoperative FLACC scale were considered to have postoperative pain and thus administered 0.5 mg/kg meperidine intravenously for analgesia.

All patients included in the study were followed up intraoperatively and postoperatively in terms of possible complications such as nausea-vomiting, laryngospasm, bronchospasm, and bleeding. The anesthetist, who performed the intraoperative follow-up of all the patients and made the postoperative PAED and FLACC evaluations, was blinded to the patients' groups.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine whether the quantitative variables conform to the normal distribution. In the comparisons of two independent groups, the two-sample independent t-test was used in the case of normally distributed variables, and the Mann-Whitney U test was used in the case of non-normally distributed variables. Descriptive statistics pertaining to quantitative variables were expressed as mean±standard deviation (SD) values in the case of normally distributed variables and as median (25th-75th percentiles) in the case of non-normally distributed variables. Spearman correlation analysis was used to determine whether there was a linear relationship between quantitative variables. Pearson's chi-squared test was used to test the assumption of independence between categorical variables. Descriptive statistics pertaining to categorical variables were expressed as frequency (n) and percentage (%). Probability (p) values of <0.05 were deemed to indicate statistical significance.

Results

A total of 97 children aged 2-10 years (mean age: 5.6±2.1 years), of whom 61 (62.9%) were boys, were included in the study. Cases (n=50) with serum 25(OH)D levels<12 ng/ml were included in group 1, and cases (n=47) with serum 25(OH) D levels≥12 ng/ml were included in group 2. Thus, the rate of vitamin D deficiency [25(OH)D<12 ng/ml] was determined to be 51.5% (50/97). The distribution of the demographic, clinical, and laboratory characteristics by the patient groups is shown in Table 1. There was no significant difference between the groups in terms of demographic characteristics, surgical procedures, duration of surgery, comorbidities, serum calcium levels, and m-YPAS scale scores (p>0.05).

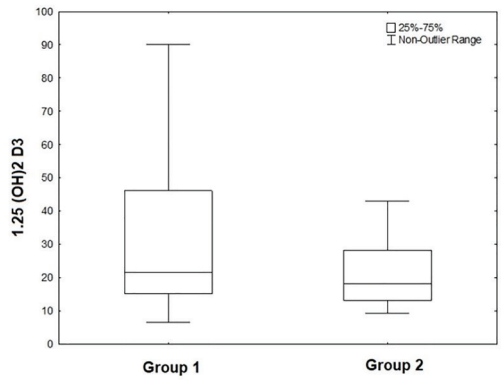

Figure 1 - Comparison of the patient groups by 1.25(OH)2D3 levels

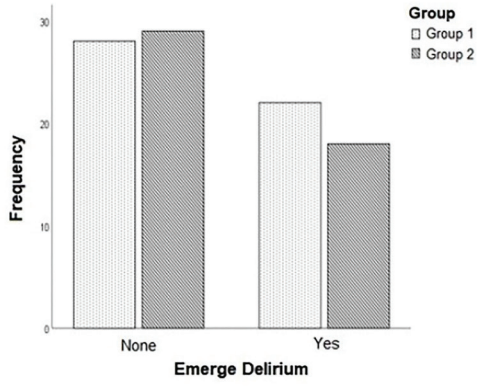

Figure 2 - Comparison of the patient groups by the presence of emergence delirium

The mean serum $1.25(OH)_2D3$ level was higher, albeit insignificantly, in group 1 than in group 2[34.4 (min.15.1, max. 46.6) pg/mL vs. 25.9 (min.13.1, max.28.2) pg/mL, p= 0.177] (Figure 1). The blood samples for serum vitamin D level measurements were taken in winter in 66% (64/97) of the children; however, no significant difference was found between the groups in terms of the season of taking the blood sample (p=0.337).

Table 1 Distribution of the demographic, clinical, and laboratory characteristics by the patient groups

	Group 1 (n=50) 25(OH)D <12 ng/ml	Group 2 (n=47) 25(OH)D ≥12 ng/ml	p
Gender (M/F)(n)	33/17	28/19	0.657
Age (year)	6 (4.7-8)	5.2 (4-7)	0.094
Body mass (kg)	22.7 (18-24.2)	19.8 (16-23)	0.151
Height (cm)	116.8±14.5	111.2±13.1	0.05
Comorbidty (Yes/No)	10/40	8/39	0.908
Comorbid Diseases Asthma n (%) Drug Allergy n (%) Allergic Rhinitis n (%) Epilepsy n (%) Hydronephrosis n (%) Chronic Bronchitis	 5(10) 2(4) 1(2) 1(2) 1(2) 0 (0)	 4(8.6) 1(2.1) 1(2.1) 1(2.1) 0(0) 1(2.1)	
Operation time (min)	38.3 (29.2-45)	39.7(30-47)	0.677
Surgical procedures Adenoidectomy n (%) Tonsillectomy n (%) Adenotonsillectomy n (%)	 33(66) 4(8) 13(26)	 32(68) 2(4.2) 13(27.6)	0.792
Serum Calcium (mg/dl)	9.5(9.3-9.8)	9.5 (9.2-9.8)	0.873
m-YPAS score	40.4 (28.3-51.6)	39 (28.3-46.6)	0.780

Data are given as median (25th-75th percentiles), mean±SD, or n (%) values.

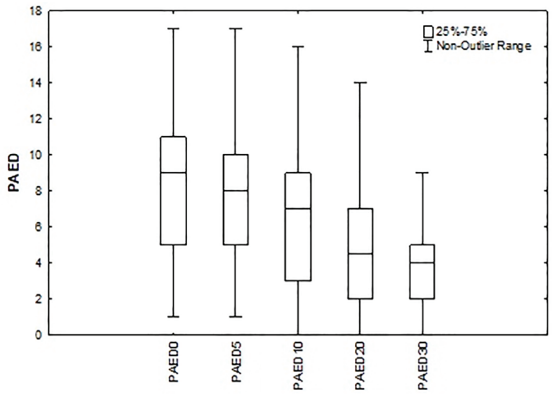

Figure 3a - PAED (0-30 min) score graph of group 1

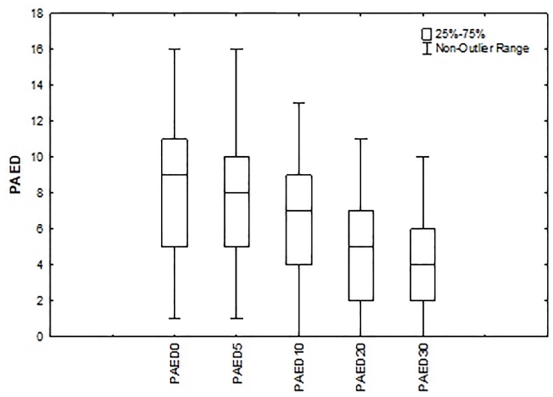

Figure 3b - PAED (0-30 min) score graph of group 2

Delirium was identified in 41.2% (40/97) of the children included in the study. 55% of the 40 children with emergency delirium were in group 1; however, there was no significant difference between the groups in terms of the number of children with emergence delirium (p=0.716) (Figure 2). There was also no significant difference between the groups in the 0-30 min PAED scores (p>0.05) (Figure 3a-3b).

Postoperative pain was detected in 37.1% (36/97) of children. There was no significant difference between the two groups in terms of postoperative pain and analgesia (p=0.692) (Table 2). At least one complication (postoperative nausea, vomiting, bleeding and broncholaryngospasm) developed in 38.1% of the children included in the study. Although there were more children with complications in group 1 (46%) than in group 2 (29.7%), the difference between the groups was not statistically significant (p >0.05).

Table 2 Comparison of the patient groups by postoperative pain

Postoperative pain	Groups		p
	Group 1 25(OH)D<12 ng/ml (n=50)	Group 2 25(OH)D≥12 ng/ml (n=47)	
Yes (n) (%)	20(40)	16(34)	0.692
No (n) (%)	30(60)	31(66)	

In group 1, there was no correlation between 25(OH)D and 1.25(OH)2D3 levels and emergence delirium and postoperative pain (p>0.05); however, there was a negative correlation between 1.25(OH)2D3 levels and m-YPAS scores (-r=0.297 p=0.034).

In group 2, there was no correlation between 25(OH)D and 1.25(OH)2D3 levels and emergence delirium, postoperative pain, m-YPAS scores, and serum calcium levels (p>0.05); however, there was a negative correlation between 25(OH)D levels and age (r=-0.378, p=0.01), height (r=-0.378, p=0.008), and weight (r=-0.348, p=0.018).

Discussion

The relationship between vitamin D levels and emergence delirium in children who underwent tonsillectomy and/or adenoidectomy was assessed in this study. Consequentially, no significant difference was found between patients with 25(OH)D deficiency and those without in terms of the presence of delirium. There are many studies on the relationship between vitamin D levels and emerge delirium in the adult patient population in the literature, most of which reported an increase in the incidence of delirium with low vitamin D levels [13,14]. However, a thorough review of the literature did not reveal any study that addressed the relationship between vitamin D deficiency and emergence delirium in the pediatric patient population.

The prevalence of vitamin D deficiency in children and adults is reported to be 30-80% worldwide. In a meta-analysis conducted in Turkey, the prevalence of vitamin D deficiency was found to be 63% [15]. Similarly, the rate of children with vitamin D deficiency [25(OH)D<12 ng/ml] was found to be 51.5% in this study.

Serum vitamin D levels vary with age. The highest and lowest vitamin D levels have been detected in the age groups of 1 to 6 years and 7 to 17 years, respectively [16]. In parallel, a negative correlation was found in this study between serum 25(OH)D levels and age.

There are contradicting results in the literature on the relationship between serum vitamin D levels and gender. In comparison, no significant difference was found between the patients with and without vitamin D deficiency in terms of gender in this study featuring the preadolescent age group.

Serum 25(OH)D levels are deemed a measure of vitamin D levels in the body. As a reason, 25(OH)D has a long plasma half-life and is found in plasma at a concentration 1000 times higher than $1.25(OH)_2D_3$ [17]. In comparison, the mean serum $1.25(OH)_2D_3$ level was found to be higher, albeit insignificantly, in group 1 than in group 2 in this study (34.4 pg/mL vs. 25.9 pg/mL, p= 0.177). Serum $1.25(OH)_2D_3$ levels are affected by serum calcium and phosphorus levels and dietary calcium intake [18]. In this context, the respective finding of this study may be attributed to the parathyroid hormone levels as a factor that might have affected the $1.25(OH)_2D_3$ levels, which, however, were not measured in this study.

High vitamin D levels are considered to protect cognitive functions, thereby preventing delirium [3]. There are many studies that addressed the vitamin D levels and the development of emerge delirium in the adult population in the literature [13,14]; however, to the best of knowledge of this study's authors, there are no comparable studies conducted with the pediatric population. Emerge delirium was identified in 41.2% of the children included in this study. In most of the studies conducted with the adult population available in the literature, a significant relationship was reported between low 25(OH)D levels and the development of delirium. In contrast, no significant relationship was found between low 25(OH)D levels and the development of delirium in this study which was conducted with a pediatric population with a mean age of 5.6 years. This discrepancy between the respective results of this study and the relevant studies in the literature may be attributed to the fact that the population of this study consisted of children aged between 2-10 years, whose brain maturation has not been completed, and that the serum $1,25(OH)_2D_3$ levels were found to be high in both patient groups studied within the scope of this study.

Although the related pathophysiology is not fully known, low vitamin D levels are thought to be associated with anxiety

[19]. As a matter of fact, many studies in the literature have reported a negative correlation between low 25(OH)D levels and anxiety [19,20]. In comparison, no significant difference was found in this study between the patients with low and normal 25(OH)D levels whose preoperative anxiety was assessed with the m-YPAS scale. This discrepancy between the respective results of this study and the relevant studies in the literature may also be attributed to the fact that the serum $1,25(OH)_2D_3$ levels were found to be high in both patient groups studied within the scope of this study.

On the other hand, negative correlations were found between m-YPAS scores and age consistent with the literature. These findings may be attributed to the greater attachment to parents, separation anxiety, and difficulty adapting to the environment experienced by the children in this age group.

Vitamin D is known to have an anti-inflammatory effect by suppressing the expression of the COX-2 enzyme, which is involved in the synthesis of prostaglandins and increasing the expression of enzymes that inactivate prostaglandins [21]. Additionally, it is known that vitamin D exerts an immunomodulatory effect by regulating the cytokines involved in pain perception and the spread of pain [22]. There are only a few studies in the literature that evaluated the relationship between postoperative pain and vitamin D levels. In comparison, no significant relationship was found in this study between the 25(OH)D levels and postoperative pain and postoperative analgesic requirement, which may be due to the fact that lesser postoperative pain is experienced in adenoidectomy and/or tonsillectomy compared to major surgeries, and that the intraoperative local anesthesia administered by the surgeon might have masked any difference that could have otherwise arisen between the groups in terms of pain.

It has been reported that the frequency of emergence delirium decreases with age in pediatric patients [23]. In parallel, in a study, it was reported that the PAED scores were highest in children aged <6 years and lowest in children aged ten years, indicating that age was a risk factor for the development of emergence delirium [24]. Similarly, a negative correlation was found in this study between the children's PAED (0-30 min) scores and their age. This result may be explained by the fact that children have less tolerance to hunger and pain and may also be attributed to the difficulties they experience in adapting to the environment and establishing communication.

Preoperative anxiety in children is thought to be associated with the development of emergence delirium [25]. In a study that included children who underwent adenoidectomy and tonsillectomy and were not premedicated, it was reported that children in the group with high anxiety levels showed more agitation symptoms in the postoperative period [25]. Similarly, a positive correlation was found in this study, which featured unpremedicated children, between preoperative anxiety measured by m-YPAS and PAED scores. This finding, taken together with the relevant findings reported in the literature, indicates that preoperative anxiety is a risk factor for the development of emergence delirium.

Inadequate treatment of pain in pediatric patients leads to the activation of the physiological and biochemical stress response, causing deterioration in many functions in the body [20]. Along these lines, it was reported in many studies conducted in a number of different surgical fields that high FLACC scores were associated with high PAED scores [26,27]. Similarly, a positive correlation was found in this study between the FLACC and PAED scores. Therefore, postoperative pain, as preoperative anxiety, also seems to be a risk factor for the development of emergence delirium.

Limitations of the Study

The prospective design of this study and the fact that it is the first study to assess the relationship between vitamin D deficiency and emergence delirium in the pediatric patient population constituted its strengths. However, there were also some limitations to this study. First, the study sample was relatively small. Secondly, serum parathormone levels could not be measured. Third, the daily calcium intake, nutritional status, clothing style, and socioeconomic status of the families of the children included in the study were not questioned. Finally, parental anxiety, which is thought to be a risk factor for emergence delirium, was not evaluated.

Conclusion

Children with vitamin D deficiency are frequently encountered in anesthesia applications. This study is the first study to evaluate the relationship between 25(OH)D levels and emerge delirium in children. Accordingly, the incidence of emergence delirium in children with 25(OH) vitamin D deficiency was not found to be high. In addition, there was no significant relationship between 25(OH)D levels and preoperative anxiety and postoperative pain. Although postoperative complications (nausea-vomiting, bleeding and broncholaryngospasm) were seen at a higher rate in the group with vitamin D deficiency, they were not significant. This study's findings suggest that using the preoperative m-YPAS scale scores would be beneficial in predicting emerge delirium and that the development of delirium can be reduced by good management of postoperative pain in pediatric patients. Further prospective, large-scale, multicenter studies are needed to be carried out in the pediatric population to corroborate the results of this study.

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A cross sectional study of knowledge, attitude and practices of medical students regarding COVID-19 in Northern India

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Abstract

Background: Practicing preventive measures essential to control the spread of infection and possessing the required knowledge, attitude and practices (KAP) towards COVID-19 could possibly affect the behavior and perception of medical students towards the disease which must be observed and studied. Studies have been conducted to assess medical students' willingness to volunteer in the ongoing COVID-19 pandemic, but not many have focused on their level of pandemic preparedness and eligibility to volunteer. Our study explored the knowledge, attitude and practices of medical students, and also helps to develop effective action plans regarding the reopening of medical colleges in India.

Material and methods: This cross-sectional observational study was done in a tertiary care center in North India on Medical and Dental undergraduate students spanning across all professional years after they gave a written informed consent. A self-designed questionnaire was established based on published literature. The study questionnaire consisted of four sections - socio-demographics of students, knowledge, attitudes/beliefs and practice-based statements about COVID-19.

Results: 238 students participated in this study and for majority (79.4%) of students the major source of information for gaining knowledge towards COVID-19 was social media. All students were aware that COVID-19 spreads through droplet infection, 94.1% responded that people with chronic illnesses were at high-risk of infection and 91.2% and 88.2% reported that fever and dry cough were the main symptoms of COVID-19 respectively. The majority of students disagreed that they would avoid isolation (n=147), and the students also disagreed on aspect of keeping the report confidential if a near one is affected (n=140). Though there were no statistically significant differences ($p<0.05$) in the level of knowledge, attitude and practices found between students studying in different professional years.

Conclusion: To conclude, the results of our study showed that major source of information for gaining knowledge for COVID-19, was through social media. The students demonstrated a satisfactory level of knowledge, attitude and practices for the prevention of COVID-19.

Key words: knowledge, attitude, practice, medical students, pandemic

Introduction

Coronaviruses (CoVs) are non-segmented single-stranded RNA viruses belonging to Coronaviridae family which are further classified into α-CoV, β-CoV, γ-CoV and δ-CoV based on their antigenicity [1-3]. α-CoV and β-CoV are responsible for causing infections ranging from common cold to croup, bronchiolitis and pneumonia in animals as well as humans [1,4,5]. Corona Viruses are primarily enzootic infections but become zoonotic when they cross the species barriers to infect humans [1,6,7]. The outbreaks of Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS)

has led to virulent infections in humans [1,6,7]. An outbreak of pneumonia of unknown origin was reported in the Chinese City of Wuhan in a cluster of patients which was identified as Covid-19 caused by severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) infections in December 2019 [8-10]. The virus was quick to spread and later was reported from many other regions of China and other countries across the world with human-to-human transmission [8,11]. World Health Organization (WHO) declared Covid-19 as a Public Health Emergency of International Concern and later declared it as a pandemic in early 2020 [8,12]. India also reported cases of Covid-19 with first case being identified in Late January 2020 which was associated with increased morbidity and mortality throughout the country. Although the case fatality in India was 3.17% which was better than the world's case fatality of 5.16% but owing to the highly populated nation, India was the worst-hit Asian country by the pandemic [13,14]. Though all age groups were affected, certain strata of the population were at high-risk of severe illness and high mortality. Patients with diabetes mellitus, hypertension, cancer, cardiac or chronic lung diseases were identified to be associated with higher morbidity and mortality [15,16]. The incubation period varied from 5 to 14 days and the patient could be contagious during initial few days in the pre-symptomatic period [15,17,18]. The most effective way to contain spread was through preventive measures which included isolation of cases, limiting travel, imposing lockdowns, regular hand washing, social distancing, wearing masks and avoid crowded places [15,19]. These preventive measures were essential to control the spread of the virus and required possession of an adequate level of knowledge, attitudes, and practices (KAP) towards covid-19 [15,20]. The entire daily routine came to a standstill which included a difficult time and situation for the education system worldwide. It not only curtailed the autonomy and independence of adults but also of students especially undergraduate students with limited experiences. This could affect their behavior and perception which needed to be studied and observed [8]. KAP Survey can help understand the perception and behavioural changes in health care workers and medical students providing the attitude of respondents towards Covid-19 [21-24]. This could further have a positive impact on friends and family of medical students as they are the most trusted source of information for them [21,25]. This further influence the surrounding community and could help in optimizing the involvement of medical students in the era of misinformation [21,26]. A KAP study done on students of different ages, batches, colleges and gender showed that all these students had adequate knowledge of modes of transmission, manifestation and prevention strategies but had limitations in their knowledge of manifestations in pregnancy. Although, students were equipped to help the frontline workers in management of Covid-19 [13]. Another study also demonstrated that behavior practices had changed but there was no significant change in attitude of the students [20]. A KAP Study demonstrated a positive correlation between the various variables [27]. Although, studies have been conducted to assess medical students' willingness to volunteer in the ongoing COVID-19 pandemic, not many focus on their level of pandemic preparedness and eligibility to volunteer. Our study explores the knowledge, attitudes and practices of medical students and helps in developing effective action plans regarding the reopening of medical colleges in India and to determine whether they are prepared to play role of a volunteer in the current crisis.

Objectives of the study: To assess the current knowledge of medical students regarding COVID-19 and explore their sources of information for the same. To understand their attitude towards reopening of medical colleges and their willingness to volunteer in the pandemic. To gain insight into the practices and precautionary measures followed by medical students to avoid personal infection. To assess the overall level of pandemic preparedness among medical students.

Material and methods

This cross-sectional observational study was done in a tertiary care center in North India on Medical and Dental undergraduate students spanning across all professional years. Only those participants were enrolled in the study who filled up the questionnaire circulated on social media and gave written informed consent. Participation of students in the study was voluntary and all questions were mandatory with each student allowed only one attempt. All undergraduate medical students (Bachelor of Medicine and Bachelor of Surgery (M.B.B.S) and Bachelors of Dental Surgery (B.D.S)) from first to final year at tertiary care center were enrolled in the study only if they were willing to give informed consent and any student of less than 18 years of age was excluded from the study.

A self-designed questionnaire was prepared based on the input from published literature. The study questionnaire consisted of two sections. Section one was designed to explore the socio-demographic profile of students and comprised of few demographic variables which included their age, gender, course, name of college etc. Section two comprised of 3 subsections, the first subsection was designed to know the major sources of information used by students to gain knowledge about COVID-19 – this subsection had 18 questions to evaluate students' in-depth knowledge and included multiple choice questions on the route of transmission, incubation period, symptoms, high-risk groups, complications, investigations, diagnosis, and current treatment regarding COVID-19 and knowledge assessing statements to be answered as True/False/Not sure. Subsection two comprised of twelve statements aimed to evaluate students' attitudes/beliefs about COVID-19. The questions on attitude were designed based on a 5-point Likert scale (1 = strongly agree, 2 = agree, 3 = neutral, 4 = disagree, and 5 = strongly disagree). The final subsection comprised of thirteen practice-based statements and was to be responded with never/sometimes or rarely/often according to the frequency with which these practices were followed by the individual. The Attitude and Practice sections were prepared based on the circumstances likely to be faced by undergraduate students. The anonymized questionnaire had been developed using Google Forms. A literature search was done with keywords like "medical students", "COVID-19", "Pandemic", "KAP", "Preparedness", "medical colleges", "volunteer", and "medical task force".

All undergraduate medical and dental students across all professionals were approached through email and social networking apps like WhatsApp and an online questionnaire was distributed. A brief introduction of the study highlighting the aims and objectives, expected duration of subject participation, benefits/risks associated with participation and informed consent were included at the beginning of the survey. Class Representatives for each academic year were involved in the process of distributing the questionnaire link to students directly to ensure prompt responses from all the batches.

A pilot study to assess the validity and reliability of the questionnaire was done among a small number of students (n = 34), and the average time taken to complete it is around 10 minutes. The feedback questionnaire was analyzed for the

coefficient of reliability by Cronbach's Alpha and gave an internal consistency of 0.831. The students' who were the part of pilot study were excluded from the study.

Statistical analysis

Feedback was expressed as a percentage of all the responses. The data was tabulated as mean±standard deviation (mean±SD), the results being analyzed using non-parametric (Chi-Square Test) and parametric (Unpaired Student 't' test and ANOVA for comparison of results of all three batches) tests. A $P<0.05$ was considered statistically significant. The data was analyzed using the SPSS Version 25.

Ethical consideration

The study was approved by Institutional Review Board (IRB) and conducted in accordance with ICH-GCP guidelines.

Results

238 students participated in this study on knowledge, attitude and practice among medical undergraduate students regarding Covid-19. Out of the 238 students, 55.9% (n=133) were females and 41.2% (n=98) were males. A majority of students – 97.1% (n=231) were pursuing MBBS course while 7 students pursuing BDS course participated in the study. Students of both MBBS and BDS spread from over first year to final year participated in the study as shown in Table 1 with maximum students of fourth/final year participating in the study.

Table 1 Year-wise distribution of students

Year	Percentage (n)
First year	14.7 (35)
Second year	29.4 (70)
Third year	23.5 (56)
Fourth/Final year	32.4 (77)

Response of KAP questionnaire by the students

Knowledge related to Covid-19 is shown in Table 2. In response to the major source of information for gaining knowledge towards the Covid-19, 79.4% (n=189) reported that social media was the major source of information. All the students answered that Covid-19 spread through droplet infection, 94.1% (n=224) responded that people with chronic illness were at high-risk for morbidity and mortality associated with infection. Most of the students stated that the incubation period varies from 2-14 days to 10-14 days with 91.2% (n=217) and 88.2 (n=210) reported fever and dry cough as the main symptom of Covid-19, respectively. There were no statistically significant differences ($p<0.05$) between the knowledge of students studying in different professional years.

Table 3 represents the questions assessing the knowledge component with the option of answers being either true, false, or not sure. 210 participants were aware that there is no current effective cure for Covid-19 with 189 participants responding that Covid-19 is transmitted even if fever is not present (n=189). All the participants responded that isolation and treatment of infected people were effective means to reduce spread of infection and asymptomatic patients can spread the disease. Though there were no statistically significant differences ($p<0.05$) in the level of knowledge between students studying in different professional years.

The response of students to the attitude aspect of the questionnaire are shown in Table 4. The questions had answers on a 5-point Likert scale rating from strongly disagree to strongly agree. The majority of students disagreed with the view that they would avoid isolation (n=147), the students also disagreed on the aspect of keeping the report confidential if a near one is affected (n=140). In response to other questions, most of the students (n=119) were ready to take all necessary precautions to prevent the spread of infection. There were no statistically significant differences ($p<0.05$) in the attitudes of students studying in different professional years.

The response of students to the practice aspect of the questionnaire is shown in Table 5. Most students practiced social distancing in public places (n=187), wore masks when outdoors (n=217), regularly washed hands (n=224) and took all the necessary precautions. There were no statistically significant differences ($p<0.05$) in the practices of students studying in different professional years.

Discussion

238 students participated in this study on knowledge, attitude and practices in medical undergraduate students regarding Covid-19 with 55.9% being females and a majority of the students 97.1% pursuing MBBS course. In response to the major source of information for gaining knowledge towards Covid-19, 79.4% reported that social media was the major source of information. All the students answered that Covid-19 spread through droplet infection, and 94.1% responded that people with chronic illness were at high risk of mortality and morbidity. Most of the students stated that the incubation period varied from 2-14 days to 10-14 days with 91.2% and 88.2 % reported that fever and dry cough were the main symptoms of Covid-19, respectively. 210 participants were aware that there is no current effective cure for Covid-19 with 189 participants responding that Covid-19 is transmitted even if fever is not present. All the participants responded that isolation and treatment of infected people were effective means to reduce the spread of infection and were aware that asymptomatic patients can spread the disease. The questions had answers on a scale rating from strongly disagree to strongly agree. A majority of students disagreed with the view that they would avoid isolation (n=147), whereas students also disagreed on the aspect of keeping the report confidential if a near one is affected (n=140). In response to other questions, most of the students (n=119) were ready to take all necessary precautions to prevent the spread of infection. Most students practiced social distancing in public places (n=187), wore masks when outdoors (n=217), regularly washed hands (n=224) and took all precautions. There were no statistically significant differences($p<0.05$) in the knowledge, attitude and practices of students studying in different professional years.

A study done in Indonesia on implementing health protocols and preventive measures influencing the KAP of Medical students as healthcare workers during the pandemic took 525 participants who responded to a questionnaire with 18 items. Most of the students demonstrated good knowledge, attitude, and practices towards COVID-19 which was not influenced by location, although, age, institution type, and institution status played a significant role. The results obtained from our study are similar as it demonstrates that most of the students possess the requisite knowledge, attitude and practices. Though the number of items in our questionnaire were more than what is used in this study, participants in our study also demonstrated an overall preparedness of the students for the Pandemic [21].

Table 2 Percentage response to knowledge aspect for Covid-19 infection where all choices were applicable.

Questions	Response in percentage (n)
1. What are the major sources of information you have used to gain knowledge towards Covid-19 pandemic? (Tick all that apply)	
a. Newspaper/Television/Radio	70.6 (168)
b. Social Media	79.4 (189)
c. Medical Professionals	61.8 (147)
d. Official Websites (Like W.H.O)	52.9 (126)
e. NGOs	14.7 (35)
f. Religious Leaders	5.9 (14)
g. Friends and Family	44.1 (105)
2. The route of transmission for Covid-19 infection is (Tick all that apply)	
a. Airborne	58.8 (140)
b. Droplet infection	100 (238)
c. Fomite borne Transmission	23.5 (56)
d. Fecal-Oral Route	14.7 (35)
e. Blood Transmission	20.6 (49)
f. Mother to Fetus	23.5 (56)
g. From Animals	8.8 (21)
3. High risk population for Covid-19 infection include (Tick all that apply)	
a. Children	58.8 (140)
b. Pregnant Women	61.8 (147)
c. People with chronic illness	94.1 (224)
d. Elderly	91.2 (217)
e. Not sure	5.9 (14)
4. The incubation period for Covid-19 is (in days)	
a. 1-5	2.9 (7)
b. 5-10	0
c. 2-14	50 (119)
d. 10-14	44.1 (105)
e. Not Sure	2.9 (7)
5. The main clinical symptoms of Covid-19 include (Tick all that apply)	
a. Dry Cough	88.2 (210)
b. Fever	91.2 (217)
c. Runny Nose	44.1 (105
d. Myalgia	26.5 (63)
e. Dyspnea	44.1 (105)
f. Sore Throat	73.5 (175)
g. Head Ache	44.1 (105)
h. Sneezing	35.3 (84)
i. Confusion	2.9 (7)
j. Diarrhea	14.7 (35)
6. The most ideal sample taken for investigation is	
a. Blood	8.8 (21)
b. Nasopharyngeal Swab	91.2 (217)
c. Serum/ Urine/ Not Sure	0
7. Which test is preferred in Covid-19 testing?	
a. Serological Test	8.8 (21)
b. Immunological Test	8.8 (21)
c. RT-PCR	79.4 (189)
d. Titration	0
e. Not Sure	3 (7)
8. Confirmative diagnostic test in Covid-19?	
a. Only through patient's symptoms	8.8 (21)
b. Detection of causative virus based on lab diagnosis	88.2 (210)
c. Through general examination	0
d. Through systemic examination	3 (7)
e. Not Sure	0
9. The complications of Covid-19 include:	
a. Pneumonia	20.6 (49)
b. Septic Shock	0
c. ARDS	14.7 (35)
d. All of the Above	50 (119)
e. Not Sure	14.7 (35)
10.How do you judge your level of knowledge about Covid-19?	
a. Sufficient	61.8 (147)
b. Inadequate	29.4 (70)
c. Not Sure	8.8 (21)

Another study done in Rajasthan, India showed the scores attained by different batches, gender, age-group and college to be comparable with satisfactory knowledge in students regarding the symptoms, mode of spread, incubation period and precautions for prevention of disease but there was limited capacity of students in the attitude and practice section for Coivd-19. The results of our study are similar as the knowledge component of students in our study was satisfactory, though, in terms of attitude and practices, our study has shown a more positive outcome [13].

Table 3 Response to knowledge aspect for Covid-19 infection with once choice option

Question	True	False	Not Sure
There currently is no effective cure for COVID-19 but early symptomatic and supportive treatment can help most patients recover from the infection	210	14	14
People with COVID-19 will not transmit the virus to others when fever is not present.	14	189	35
COVID-19 is caused by a virus, so antibiotics do not work. Antibiotics should not be used as a means of prevention or treatment of COVID-19. They should only be used as directed by a physician to treat a bacterial infection	140	49	49
Not all people with COVID-19 develop severe cases, only older people with serious chronic illnesses like lung/heart diseases and diabetes have increased risk of developing more serious complications of COVID-19.]	182	28	28
Isolation and treatment of people who are infected with the COVID virus are effective measures to reduce spread of virus	238	0	0
Asymptomatic carriers in subclinical stage can spread the disease.	210	7	21
Mild cases of COVID-19 that improve in few days on its own need not be isolated.	42	175	21
Do you have access to any helpline number to contact in case you suspect you or someone you know had COVID-19 OR to obtain information?	182	21	7

Table 4 Response to Attitude aspect for Covid-19 infection with once choice option

Question	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
If I got infected, I would do anything to avoid isolation.	147	49	21	7	14
If I got infected , I will be extremely stressed of the way the health-workers people in hospital, hospitalization process will deal with me	77	63	47	28	21
If somebody in my family were to get COVID-19, I would want it to remain private or a secret.	140	42	28	7	21
When called upon, I will willingly attend physical offline classes, practical's and clinical postings in the hospital if my college reopens.	56	28	70	21	63
When called upon, I will willingly participate in the frontline of COVID-19 pandemic response.	21	21	63	56	77
I will go into institutional quarantine if I come in contact with a patient of COVID-19.	35	28	28	63	84
I think I am capable to endure such a public health emergency with proper training.	14	28	56	63	77
I will readily take all necessary precautions (like wearing PPE) and maintain sanitation to prevent personal infection or spread of the infection.	14	14	35	56	119
I will not go for any postings in a hospital where COVID-19 patients are treated.	35	42	63	56	42
I will not go for any postings in a hospital without a clear COVID-19 infection control isolation policy.	14	14	63	63	98
I fear self-infecting or infecting family members and high risk groups during COVID-19 duty.	21	14	49	70	84
If a person known to me(neighbor/relative) gets infected with COVID-19, I will help him/her in my best ability.	14	14	35	49	126

Table 5 Response to Practice aspect for Covid-19 infection with once choice option

Question	Never	Sometimes	Often
I maintain a social distance of 1 meter at public places.	0	49	189
I wear a mask whenever I go outside.	7	14	217
I wash and/or reuse my mask.	42	56	140
I refrain from touching my face and shaking hands.	14	56	168
I regularly wash my hands with soap and water.	7	7	224
I use hand sanitizers and disinfectants more frequently.	7	21	210
I avoid unnecessary travel and public gatherings.	0	49	189
In case I am not wearing a mask, I cover my mouth or nose during a cough or sneeze with elbow/a tissue.	0	28	210
I listen and follow the direction of state and local authorities.	7	21	210
I closely monitor my personal physical health and that of other people around me.	0	21	217
I persuade people around me to follow the precautionary guidelines.	7	14	217
In order to prevent contracting COVID-19, I take vitamin supplements/herbal products/traditional medicines.	35	84	119
I always carry a hand sanitizer while going out of the house.	0	28	210

Another study was done in Jordan and one done in Vietnam on a sample of medical students to assess their knowledge, attitude and practices towards Covid-19 in six medical colleges demonstrated that the main source of knowledge for students was social media and online search engines, with less preference for medical search portals. The students had satisfactory knowledge of the route of transmission but limited response was obtained for droplet infection as the main source of transmission. The students also showed a satisfactory level of attitude and practice for the pandemic. The results of our study are quite similar as students in our study demonstrated a satisfactory level of KAP as well and their main source of information was through social media [1,15].

One more study done in India for assessing the KAP of Medical Students showed no significant relationship in terms of knowledge, attitude and practices between different age groups and religions, though this study did show a significant impact of gender on the practice score with the majority of participants possessing satisfactory knowledge, attitude and practice regarding the pandemic. The results of this study are similar to our study which showed satisfactory knowledge, attitude and practice though there was no difference in terms of gender in the participants [20]. Another study showed that undergraduate students in response to the Covid-19 outbreak had acquired the necessary knowledge, positive attitude and proactive practices but their scores significantly varied by gender, major and school type. This is different from our study as there was no difference in terms of gender, year or religion in our study [8].

Another study done in India demonstrated that 25% of students had low- to moderate-level knowledge, had a negative attitude and undesirable practices in preventing the pandemic (Covid-19) thereby suggesting approaches to enhance KAP amongst medical students. The results of our study are different from this study as our study demonstrated a satisfactory level of knowledge, attitude and practices among medical students regarding the pandemic [27].

There are a few limitations of our study, firstly is the sample size as the participation was voluntary and through online sessions, so the response after students joining back and after appropriate training might depict better scores and better participation in the KAP study. Secondly, the study only provides a snapshot of the KAP level of students, training the students regarding the pandemic could yield different results.

To conclude, the results of our study showed that the major source of information for gaining knowledge towards Covid-19 was through social media and students demonstrated a satisfactory level of knowledge, attitude and practices for the prevention of Covid-19. Though awareness campaigns and proper training can equip these students to handle the situation appropriately.

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Retrospective examination of endometrial sampling results in women with abnormal uterine bleeding

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Abstract

Background: Abnormal uterine bleeding (AUB) is among the most significant and frequent causes of admission to the gynecology outpatient clinic. AUBs may manifest as the earliest sign of endometrial cancer. For the early detection and treatment of endometrial cancer, careful examination of AUBs is crucial.

Aim: The study was conducted to retrospectively evaluate the histopathological results of probe curettage materials applied to women who applied to the gynecology clinic for AUB and were hospitalized with the complaint of AUB.

Material and Methods: In the retrospective study planned between 2020 and 2021, all endometrial biopsies from 638 women with AUB reported to the gynecology clinic were reviewed and analyzed. The data were obtained from the archives of our hospital's gynecology and obstetrics clinic and pathology clinic.

Results: 638 cases were analyzed. The mean age of the patients was 47.94±9.53 years. Malignant pathology was detected in 20 cases (3.13%). Nineteen of these cases were seen in postmenopausal women. The most common pathology was found to be benign polyps at a rate of 19.91%. It was the most common benign pathology in women pre- and postmenopausal. Adenocarcinoma was detected in 13 (2.03%) postmenopausal cases while in only 1 (0.16%) of the premenopausal women.

Conclusion: Since more malignant pathologies are observed in postmenopausal women, women with asymptomatic or AUB complaints should be carefully monitored, and endometrial evaluation should not be skipped. Endometrial biopsies are valuable in the early detection of pre-cancerous and cancerous endometrial lesions, especially in postmenopausal women.

Key words: endometrial cancer, abnormal uterine bleeding, endometrial carcinoma

Introduction

One of the most important and common reasons for admission to the gynecology outpatient clinic is abnormal uterine bleeding (AUB) [1,2]. AUB reduces the quality of life by imposing unorthodox physical, emotional, sexual, social, and financial responsibilities on women [3]. AUB is abnormal changes in time, frequency, and volume patterns in blood flow in the menstrual cycle [3]. AUBs are shared among the reasons women apply to gynecology outpatient clinics in the pre and post-menopausal period during reproductive age. Approximately one out of every three applications is due to AUB [3]. The prevalence and prevalence of 10-30% in women of reproductive age led researchers to a common terminology for defining and classifying AUBs [2]. This standard classification was made by The International Federation of Gynecology and Obstetrics (FIGO) in 2011. This classification is named PALM-COEIN by FIGO [2–4]. In this classification, PALM is classified as a polyp, adenomyosis, leiomyoma, malignancy, and hyperplasia; and the coin is classified as coagulopathy, ovulatory dysfunction, endometrial

causes, iatrogenic and not yet classified [4]. Although a common terminology has been acquired for healthcare professionals in the AUB after this classification, it is of great importance how reproductive women perceive abnormal uterine bleeding, with which complaints they come to the clinic, and in what period they come [2].

AUBs are often the first symptom of endometrial cancer. Endometrial cancer is the most common gynecological malignant tumor in developed countries and ranks second after cervical cancer in developing countries [5]. Careful evaluation of AUBs is of great importance for early diagnosis and treatment of endometrial cancer. Especially in the pre and post-menopausal period, women who apply with the complaint of AUB should be carefully evaluated [6].

Diagnosis is made by probe curettage in AUBs. After the probing process, a diagnosis is made according to the PALM-COEIN classification, and the follow-up and treatment process begins. Age, body mass index (BMI), and systemic diseases [such as diabetes and hypertension] of the patients pose a risk in terms of the malignancy potential of polyps. In addition, a relationship was found between menopausal status, hormonal replacement therapy, history of breast cancer, tamoxifen use, and development of malignancy from polyp [7–9]. Although many factors affect the diagnosis, the fact that the woman is in the post or premenopausal period increases both the rate of malignancy and the likelihood of developing endometrial cancer. When the literature is examined, it is seen that the malignancy rates of women in the premenopausal period who apply with the complaint of AUB are 0.4%. In comparison, the malignancy rates of women in the postmenopausal period are 7%. In particular, it is seen that experiencing AUB after menopause has a 17.5-fold effect on the malignant outcome rate.

Similarly, as a result of the diagnosis in the literature, it was found that the incidence of hyperplasia was 2.5 times higher in the postmenopausal group than in the premenopausal group, the incidence of the endometrial polyp was four times higher, the incidence of proliferative endometrium was 0.38 times lower, and 50% of women in the postmenopausal period were diagnosed with secretory endometrium [3,10]. In line with these results, timely AUB screening is essential in both endometrial cancer and eliminating women's complaints. The results of probe curettage performed due to AUB in clinics reveal the severity of the condition [2,3,7,10].

Our study aims to retrospectively evaluate the histopathological results of probe curettage materials applied to women who applied to the clinic due to AUB and were hospitalized with the complaint of AUB.

Material and methods

The data of 638 patients admitted to the Obstetrics and Gynecology Clinic of Karabük University Training and Research Hospital between 2020 and 2021 or hospitalized in the puerperal ward due to abnormal vaginal bleeding were retrospectively analyzed. Age, the premenopausal and postmenopausal status of 638 patients, histopathological results of the unit sending for biopsy, and probe curettage materials were recorded. Histopathological results were classified into four groups: benign, premalignant, malignant pathology, and inadequate sample.

Inclusion criteria: applying to the clinic due to abnormal uterine bleeding, being in the menopausal period.

Exclusion criteria: not being in the menopausal period, insufficient material received.

Data analysis

Statistical analysis of the study was performed with SPSS 20 computer software (SPSS, Chicago, United States). Since the Skewness and Kurtosis values of the data remained within the +2.0/-2.0 limit range, it was seen that the data showed normal distribution [11]. Chi-square [$\chi 2$] test was performed to compare categorical characteristics. Frequency, Percentage, Average, and Standard deviation of descriptive statistical methods were used to evaluate the study data. One-Way ANOVA was used in all three and above comparisons in the study. Bonferroni-corrected Tukey HSD comparison was performed to determine which group caused the difference in evaluating the significant difference in the groups. The data were evaluated at a 95% confidence interval and $p<0.05$ significance level.

Results

The data of 638 patients who underwent probe curettage due to abnormal vaginal bleeding in the clinic between 2020 and 2021 were evaluated. The results evaluated were divided into premenopausal and postmenopausal periods and analyzed. The mean age of the women included in the study was 47.94 ±9.53. It was observed that 46.9% (n=299) of the women were in the premenopausal period, and 53.1% (n=339) were in the postmenopausal period. When the units where the pathologies were taken were evaluated, it was seen that 35.3% (n=225) were in the puerperal-perinatology service, and 64.7% (n=413) were in the obstetrics and gynecology outpatient clinic (Table 1).

Table 1 Characteristics of the data included in the study

Features		Mean±Ss	Min-Max (Median)
Age		47,94±9,53	24- 87 (47)
		n	%
Menopause Status	Premenopausal Period	299	46,9
	Postmenopausal Period	339	53,1
Unit where pathology was taken	Postpartum-Perinatology Service	225	35,3
	Obstetrics qnd Gynecology Polyclinic	413	64,7
Total		638	100,0

According to the histopathological findings of the patients due to ASF, 78.4% (n=500) were benign, 3.1% (n=20) were malignant, 3.0% (n=19) were premalignant, and 15.5% (n=99) were insufficient. Pathology results according to benign, premalignant, and malignant lesion types are given in Table 2 as percentages and frequency.

The distribution of pathology results by sending unit is given in Table 3. There was a difference between the distribution of pathology results of the patients hospitalized in the Postpartum-Perinatology department compared to the patients admitted to the outpatient clinic ($p <0.05$) (Table 3).

The relationship between age distribution and pathology result distribution of the patients was explained by One-Way Analysis of Variance (ANOVA). According to the analysis results, a significant difference was found between the patient's age distribution and pathology result distribution ($p<0.05$). Between which groups these differences were determined by the Tukey test in post-hoc analysis [homogeneity of variances $p= 0.000$; $P<0.05$) (Table 4). According to the analysis results, pathology results show that the mean age of patients with benign malignancies is lower than those with malignancies and inadequacies. In comparison, the mean age of patients with malignancies is higher. These differences were also statistically significant ($p<0.05$).

Table 2 Histopathological findings of the data included in the study

Features		Mean±Ss	Min-Max (Median)
		n	%
General Histopathological findings	Benign	500	78,4
	Premalignant	19	3,0
	malignant	20	3,1
	Insufficient Sample	99	15,5
Characteristics of Histopathological Findings			
Benign Pathologies	Bening	145	22,7
	Bening, Atrophic Endometritis	2	0,3
	Bening, Irregular Proliferation	56	8,8
	Bening, Irregular Secretory endometrium	1	0,2
	Bening Polyp	127	19,9
	Bening, Proliferative Endometritis	51	8
	Bening Polyphosis Development	47	7,4
	Bening, Proliferative Polyp	2	0,3
	Bening, Secretory endometrium	69	10,82
Premaling Pathologies	Premaling, Atypical Secretory Endometritis	1	0,2
	Premalingn Simple hyperplasia without atypia	15	2,4
	Premaling Complex Atypia	3	0,5
Malignant Pathologies	Malignant Endometrioid Adenocarcinoma	14	2,2
	Maling Bercan Carcinoma	2	0,3
	Maling Endometrial Neoplasia	2	0,3
	Maling Carcinoma	2	0,3
	Insufficient Sample	99	15.5
Total		638	100,0

Pathology results according to the menopausal period of women are given in Table 5. According to the analysis results, it was found that the pathology results of women who had AUB in the postmenopausal period were higher than those who had AUB in the premenopausal period (Table 5). When the malignant pathology results were examined, it was found in 1 (5%) case in the premenopausal group and 19 (95%) cases in the postmenopausal group (Table 5).

The histological pathology distributions of the cases according to their menopausal status are given in Table 6. No comparison was made in the analysis results, and only which pathology was seen in which period was examined. In line with these results, the most common result in both pre and postmenopausal women was found to be benign pathology. In addition to these findings, more malignant pathologies were found in postmenopausal women. He incidence of adenocarcinoma in postmenopausal women was 13 times higher than in premenopausal women (Table 6).

Discussion

Abnormal uterine bleeding is the most common reproductive age problem in women [8]. AUB should be evaluated quickly and carefully for endometrial cancer in women older than 40 years of age, especially in postmenopausal women. One-third of outpatient visits to the gynecology and obstetrics outpatient clinic and more than 70% of the patients are AUB [8]. It is seen that OCD increases with age and is effective in the transformation of age into a risky condition, especially endometrial cancer [3,6,8,12]. In the study by Şahin et al., in order to compare the endometrial thickness and histopathological results measured by transvaginal ultrasonography in premenopausal patients with abnormal uterine bleeding, it was observed that the mean age of women was 40 years and above, and they were in the premenopausal period [6]. Aker et al. examined the endometrial results in women with abnormal uterine bleeding, and in their study involving 765 cases, it was observed that the mean age of women was 43.14±7.92 in the premenopausal group and 60.7±7.88 in the postmenopausal group [3].

Table 3 Distribution of pathology results according to the unit that sent the biopsy

	Benign n (%)	Permalignantn (%)	Malignant n (%)	Insufficientn (%)	Total n (%)*	p
Obstetrics and Gynecology Clinic	313(%75.8)	13(%3.1)	9(%2.2)	78(%18.9)	413(%100)	0.004
Postpartum-Perinatology service	187(%83.1)	6(%2.7)	11(%9.3)	21(%9.3)	225(%100)	
Total	500(%78.4)	19(%3.0)	20(%3.1)	99(%15.5)	638(%100.0)	

* Row percentage is taken. χ^2=13.08 Sd=3

Table 4 ANOVA results regarding the significance of the difference between age and pathology outcome distributions

Pathology outcome distributions		n	x̄	ss	F	P	Difference between groups
Patient age	(1) Benign	500	46.76	8.383	18.423	0.000	1<3 p=0.000
							1<4 p=0.005
	(2) Permalign	19	50.00	12.529			2 <3 P=0.031
	(3) Malignant	20	60.05	8.500			3>1 p=0.000
	(4) Insufficient	99	51.02	11.930			3>2 p=0.031
							3>4 p=0.002
							4>1 p=0.005
							4<3 p=0.002
Homogeneity of variances = 0.000							

Table 5 Pathology results by menopausal period

Menopause Status	Benin		permaligin		malignant		Insufficient		Test Statistic*	p
	n	%	n	%	n	%	n	%	28,755	0,00
Premenopausal	259	51,8	8	42,1	1	5,0	31	31,3		
Postmenopausal	241	48,2	11	57,9	19	95,0	68	68,7		

*chisquare

Table 6 Histopathological findings of the cases according to their menopausal status*

Characteristics of Histopathological Findings	Menopoz Durumu					
	Premenopause Period			Postmenopause Period		
	n	Satır%	Sütun %	n	Satır%	Sütun %
Bening	69	47,59	23,1	76	52,41	22,4
Bening, Atrophic Endometritis	0	0,00	0,00	2	100,00	0,6
Bening, Irregular Proliferation	24	42,86	8,8	32	57,14	9,4
Bening, Irregular Secretory endometrium	1	100,00	0,3	0	0,00	0,00
Bening Polyp	61	48,03	20,4	66	51,97	19,5
Bening, Proliferative Endometritis	31	60,78	10,4	20	39,22	5,9
Bening Polyphosis Development	19	40,43	6,4	28	59,57	8,3
Bening, Proliferative Polyp	2	100,00	0,7	0	0,00	0,00
Bening, Secretory endometrium	52	75,36	17,4	17	24,64	5,0
Premaling, Atypical Secretory Endometritis	1	100,00	0,30	0	0,00	0,00
Premalingn Simple hyperplasia without atypia	7	46,67	2,30	8	53,33	2,40
Premaling Complex Atypia	0	0,00	0,00	3	100,00	0,90
Malignant Endometrioid Adenocarcinoma	1	7,14	0,30	13	92,86	3,80
Maling Bercan Carcinoma	0	0,00	0,00	2	100,00	0,60
Maling Endometrial Neoplasia	0	0,00	0,00	2	100,00	0,60
Maling Carcinoma	0	0,00	0,00	2	100,00	0,60
Insufficient Sample	31	31,31	10,4	68	68,69	20,1

*Comparison analysis was not performed in the table, only the histopathological findings between groups were examined.

In a retrospective study by Çelik and Güngör involving 705 cases, it was observed that the mean age of women was 46.93±9.04 years [12]. n our study, it was observed that the mean age of the women who came with the complaint of AUB was 47.94±9.53 years, 46.9% in the premenopausal period, and 53.1% in the postmenopausal period. These findings are similar to the average age of women with the complaint of AUB in the literature [3,6,12].

AUB is among the early symptoms of endometrial cancer. Women presenting with OCD anywhere in the premenopausal or postmenopausal period should be closely evaluated for cancer incidence [6,13–15]. When the literature was examined, it was seen that women who came with the complaint of AUB were diagnosed with endometrial cancer in the range of 6.2-15.2% [10,15–18]. In the studies in our country, this rate was found to be between 0.3-5% [3,6,12]. Bosch et al. investigated different endometrial and other intracavitary pathologies in women who presented with abnormal uterine bleeding using the International Endometrial Tumor Analysis (IETA) terminology before and after menopause and found endometrial cancer in 137 (6.2%) of women in their study involving 2856 women [15]. In a prospective observational study by Saccardi et al. Investigating the clinical relationship between the endometrial thickness (ET) and abnormal uterine bleeding (AUB) on the risk of endometrial cancer [EC] in a cohort of postmenopausal patients undergoing diagnostic hysteroscopy and endometrial biopsy, 16 (15.2%) of 105 women with only complaints of AUB were diagnosed with an endometrium [17]. In the study of Şahin et al., it was observed that 0.3% (n=2) of the women who applied to the clinic with AUB were diagnosed with endometrial cancer [6]. In the study of Aker et al., it was found that 5% (n=12) of women who applied with AUB were diagnosed with endometrial cancer [3]. In the studies of Çelik and Güngör involving 705 women, endometrial cancer was found in 2.4% (n=17) and other types of carcinomas in 0.4% (n=4) [12]. In the study of Sufia et al., in which 6458 Saudi women who applied to the clinic with AUB hemorrhage in the last 13 years were examined, it was observed that 1.88% (n=122) of women were diagnosed with endometrium [18]. In our study, it was observed that 3.1% (n=20) of the women who applied to the clinic with AUB were diagnosed with malignancy, and this result is consistent with the literature. Endometrial cancer or malignant diagnosis in AUBs increases with increasing age and is more common in the postmenopausal period [18]. When the literature is examined, it is seen that women diagnosed with malignancy are in the postmenopausal period or over 50 years of age [3,12,16,18]. This result is consistent with the literature and in our study, 11 of 20 women diagnosed with malignancy were found to be in the postmenopausal period.

As the unmet effect of estrogen continues with age, changes in the endometrium cause an increase in endometrial glands, and this increase causes endometrial hyperplasia without simple atypia [12]. Our study observed hyperplasia without Premalingn Simple atypia in 15 cases. 46.67% (n=7) of these cases were seen in the premenopausal period and 53.33% (n=8) in the postmenopausal period. When the literature is examined, the incidence rates of hyperplasia without simple atypia differ [3,12,15,18]. In the study of Çelik and Güngör, hyperplasia without simple atypia was 66.7% under the age of fifty, while it was 33.3% in women aged fifty and over [12]. In the study of Aker et al., hyperplasia without simple atypia was observed in 32 (4.2%) cases, and only two were in the postmenopausal period [3]. In the study of Bosch et al., the rate of hyperplasia

without simple atypia was found to be 6.7% (n=148), 66.89% (n=99) of 148 women were in the premenopausal period, and 33.10% (n=49) were in the postmenopausal period [15]. In the study of Sufia et al., simple atypical hyperplasia was observed in 254 (3.9%) cases; 18 (2.6%9 of these cases were under 40 years of age, 152 [3.4%] were between 40-55 years of age, and 84 (6.5%) were over 55 years of age [18]. In our study, hyperplasia without simple atypia was observed more in the postmenopausal period. The reason for this is thought to be the difference in interpretation between simple atypical endometrial hyperplasia and endometrial polyp in the differential diagnosis.

Endometrial polyp is one of the common causes of AUB [18,19]. This rate varies between 8-18% in studies [3,12,15,18]. The endometrial polyp was 33% in the study of Aker et al.; 34.5% in the study of Çelik and Güngör; 9.5% in the study of Kucur et al.; 18.3% in the study of Sufi et al. and 34.6% in the study of Bosch et al. [3,12,15,18,20]. In our study, the polyp was detected in 20.22% of the cases.

Endometrial adenocarcinoma [endometrial cancer] is also encountered in women who apply to the clinic due to AUB. The most important clinical symptom of endometrial cancer, especially in postmenopausal women, should be monitored very carefully, and the follow-up of these women should not be missed. The probability of adenocarcinoma in women presenting to the clinic with the complaint of AUB varies between 0.5-5%. These rates were found to be higher in postmenopausal women [3,12,15,18,20]. n the study of Çelik and Güngör, the rate of adenocarcinoma was 2.4% (n=17), and 13 of these cases were observed in women aged 50 and over [12]. In the study of Kucur et al., adenocarcinoma was observed in 6 cases (0.8%), and 4 of these cases were found to present with bleeding complaints in the postmenopausal period [20]. In the study of Aker et al., malignant pathology was detected in 12 cases (5%), and 9 of these cases were in the postmenopausal period [3]. In the study of Sufi et al., adenocarcinoma was detected in 86 cases (1.88%), and it was observed to be between the ages of 37-80 [18]. In the study of Bosch et al., endometrial cancer was observed in 137 cases (4.79%), and 127 of these cases were found to be in the postmenopausal period [15]. In our study, adenocarcinoma was observed in 14 cases (4.19%), and 13 of these cases were found to be in the postmenopausal period. Our results are consistent with the literature and have shown that women in the postmenopausal period require careful clinical examination of OCD complaints.

Limitations of the study

Conducting the study in a single center and in a single province and including the data of the last two years constitute an important limitation. The results of the study may form a basis for further studies in the field of investigating the outcomes of patients suffering from abnormal uterine bleeding in Karabuk and elsewhere.

Conclusion

Our study showed that endometrial cancer or malignant pathology increased with age. Mainly, the presence of AUB in the postmenopausal period was found to be an important differential diagnosis for defining endometrial cancer. It is essential not to make an advanced differential diagnosis for endometrial cancer and to perform endometrial sampling in postmenopausal women experiencing AUB or metrorgy.

Ethical board approval

University Scientific Non-Interventional Clinical Research Ethics Committee (protocol date and number: 25.04.2022 and Number: E-77192459-050.99-123196 Decision No: 2022/896).

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Role of pyroptosis in COVID-19

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Abstract

Objectives: In this study, it was investigated the relationship between of gasdermin-D, caspase-1, IL-1β and NLRP3, which are biomarkers that play an important role in the pyroptosis and COVID-19.

Material and methods: This study was carried out with 58 participants, 28 (48.28%) of whom were diagnosis of COVID-19, and 30 (51.72%) of whom were healthy volunteers (control group).

Results: There were no statistically significant differences between the gasdermin-D, caspase-1, IL-1β and NLRP3 levels as a result of all statistical comparisons performed. However, IL-1β values both at the discharge period and at hospitalization period were considerably higher than those of control group. At discharge period, IL-1β values of the patients with severe COVID-19 category had higher than moderate patients, and the patients with moderate than the patients with the mild patients.

Conclusion: It was observed that IL-1β, which is one of the cytokines released as a result of cell death in the pyroptosis mechanism, was higher in the COVID-19 patients both the hospitalization and discharge periods compared to the control group. Although not statistically significant these results could support the relationship between the pyroptosis and COVID-19.

Key words: COVID-19, interleukin-1β, pyroptosis

Introduction

Coronaviruses are enveloped RNA viruses belonging to the beta-coronavirus genus in the *Coronaviridae* family [1]. After the pneumonia clustering detected on 31 December 2019 was determined to be caused by a new Coronavirus never before seen in humans, this Coronavirus was defined as SARS-CoV-2 [2]. SARS-CoV-2 has been described as the 7th Coronavirus, that is pathogenic in humans [1]. Coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO) on March 11, 2020 [3]. The disease has spread to more than 220 countries the worldwide. The number of cases reached 515 million and deaths reached 6,254,140 worldwide [4].

While the disease may progress asymptomatically, it can also be seen in respiratory failure requiring mechanical ventilation, sepsis, septic shock and multiple organ failure [5]. COVID-19 is characterized by an abnormal host immune response, manifested by high blood cytokine, chemokine, and C-reactive protein (CRP) levels [6]. And it has been shown in many previous studies that COVID-19 can have multi-system involvement (cardiac, gastrointestinal, etc.) [7-14].

In addition to known cell death mechanisms such as necrosis, autophagy and apoptosis, a new type of cell death, the pyroptosis, has been defined in recent years. Pyroptosis is a type of programmed and inflammatory necrosis that occurs due to caspase (cysteinyl aspartate specific proteinase) activation and pore formation in the cell membrane conducted by the gasdermin protein

family. Pyroptosis results lytic cell death accompanied by the release of various inflammatory factors by inducing amplification of the cascade and of the inflammatory response. In addition, it is an important immune defense mechanism in the body that resists the invasion of external pathogens and plays a role in perceiving internal pathogenic signals in cells [15]. Various evidences have been shown to support that in severe COVID-19 cases, there are inflammasome activation, the pyroptosis and their critical roles. It was found that the pyroptosis is associated with caspase-1 activation, gasdermin-D (GSDMD) cleavage and increased levels of proinflammatory cytokines in primary monocytes and macrophages of COVID-19 patients [16-18]. In this study, it was investigated the relationship between of GSDMD, caspase-1, Interleukin-1β (IL-1β) and NOD-like receptor family pyrin domain-containing 3 (NLRP3), which are biomarkers that play an important role in the pyroptosis and COVID-19.

Material and methods

Study population and participant groups

The patients over 18 years of age, who were diagnosed with COVID-19 as a result of clinical signs, symptoms and laboratory tests and who were hospitalized at the COVID-19 ward of Harran University Medical Faculty Hospital and the control group consisting of healthy volunteers without any symptoms or underlying disease included in the study. The patient group consisted of the participants were diagnosed with a positive real-time reverse transcription-polymerase chain reaction (rRT-PCR) in addition to various symptoms and findings and were followed up in the hospital. The patients were grouped grounded on the "COVID 19 (2019-nCoV Disease) Guidelines" published by the Turkish Ministry of Health. According to this guideline, patients diagnosed with COVID-19 were divided into four subgroups; Group 1 was classified as mildly symptomatic, Group 2 as symptomatic (radiological involvement in addition to fever and respiratory symptoms), Group 3 as symptomatic (dyspnea, oxygen saturation ≤93% at rest) and Group 4 as critically ill (respiratory failure, clinical shock or organ failure requiring mechanical ventilation) [19].

Biochemical analyses

Blood samples were collected from both COVID-19 patients and healthy volunteers. Serum samples were obtained by centrifugation of the blood samples taken at 3500 rpm for 10 minutes and these serum samples were stored in the refrigerator at -80˚C until the day of the study. GSDMD (Gasdermin D Elisa Kit (BT Lab Catalog no: E6838Hu)), caspase-1 (BT Lab; E2248Hu), IL-1β (Interleukin 1 Beta; BT Lab E0143Hu) and NLRP3 (Human Nlr Family Pyrin Domain, BT Lab E3886Hu)) levels were performed according to the commercially purchased ELISA kit protocol. After adding 100 µl of a serum sample to the 96 plates in the kit, it was incubated at 37° C for 90 minutes. After incubation, the plate was emptied, washed twice with a washing solution, and dried. 100 µl Biotin-labeled antibody was added and incubated at 37°C for one hour. After incubation, the plate was drained and washed three times with a washing solution and dried. 100 µl HRP-Streptavidin Conjugate was added and incubated at 37°C for 30 minutes. After incubation, the plate was drained and washed five times with wash solution and dried. Then 90 µl TMB Substrate was added and incubated at 37°C in the dark. 50µl of Stop Solution was added after color formation was observed. Data were obtained by reading the plates in a microplate reader (Biotek-Cytation-1) at 450 nm absorbance.

Statistical analyses

SPSS version 22.0 was employed in the statistical analysis (SPSS Inc., Chicago, IL). G*Power v3.1.9.4 was implemented to conduct a power analysis in order to determine the sample size. There were four ways to sum up descriptive statistics: number, percentage, mean, and standard deviation (S.D). Continuous variables were examined to see if they adhered to the normal distribution using the Kolmogorov-Smirnov test. With the help of the paired samples t-test and the two independent sample t-test, continuous variables with the normal distribution were examined. The variables that did not exhibit a normal distribution were subjected to the Mann-Whitney U test and the Wilcoxon sign test. For comparing more than two continuous variables, use the Kruskal-Wallis test. For all statistical tests, p 0.05 was accepted as the significance level.

Results

A total of 58 participants, including 28 (48.28%) COVID-19 patients hospitalized in the COVID-19 ward and 30 (51.72%) healthy controls, were included in the current study.

15 (53.6%) of the patients in the COVID-19 group were female and 13 (46.4%) were male. 15 (50%) of the patients in the control group were female and 15 (50%) male. 7 (25%) of the patients were categorized in Group 1, 14 (50%) in Group 2 and 7 (25%) in Group 3. There were no patients in Group 4.

The most common complaints of COVID-19-infected patients at the time of admission were weakness-fatigue (n=25, 89.3%), cough (n=23, 82.1%), muscle-joint pain (n=20, 71.4%), shortness of breath (n=19, 67.9%) and fever (n=13, 46.4%). Comorbidities were present in 15 (53.6%) of COVID-19-infected patients. The most common comorbidities were diabetes mellitus (n=7, 25%) and hypertension (n=6, 21.4%). Comparison of the laboratory parameters of the patients during hospitalization and discharge periods showed statistically significant differences in platelet, lactate dehydrogenase (LDH), CRP, alanine aminotransferase (ALT), urea, and creatinine levels (Table 1).

However, we found no statistically significant difference between GSDMD, caspase-1, IL-1β, and NLRP3 values during hospitalization and discharge periods. In addition, there was no statistically significant difference between the biomarkers of hospitalized COVID-19 patients and the biomarkers of the control group. However, it was observed that all of four biomarkers, more prominently at IL-1β level, were found to be higher in the patient group than the control group (Table 2).

The Kruskal-Wallis was performed to detect whether difference of GSDMD, caspase-1, IL-1β and NLRP3 levels were statistically significant between the COVID-19 patient groups. As a result of the test, it was determined that there were no statistically differences between the biomarkers according to the patient's groups. Although it could not be determined the significant differences, there were remarkable differences between the means of IL-1β values of the patient's groups. It was concluded that the reason of not determining the statistically significant differences was that the standard deviations were high (Table 3).

In the COVID-19 patient group, no statistically significant difference was found between the GSDMD, IL-1β, caspase-1, and NLRP3 values of patients with lymphopenia, leukopenia, and neutropenia at hospitalization and discharge. However, in patients with lymphopenia and leukopenia, while IL-1β and GSDMD levels increased at the discharge period, it was observed that caspase-1 and NLRP3 levels decreased (Table 4).

Table 1 Laboratory parameters of COVID-19 infected inpatients at admission day and discharge day

Parameters	Admission day (n=28)		Discharge day (n=28)		p
	Mean	S.D	Mean	S.D	
Leucocyte (cells/ mm3)	5835.67	2390.26	7060.42	3333.037	0.064
Neutrophil (cells/ mm3)	4206.67	2247.06	5283.75	3027.29	0.088
Lymphocyte (cells/ mm3)	1139.67	552.98	1516.79	950.75	0.079
Platelet (cells/mm3)	204.42	74.13	300.46	160.03	0.001
HGB (g/dL)	13.80	1.68	13.44	1.95	0.132
PT (seconds)	11.79	1.87	11.77	2.18	0.755
INR	0.95	0.15	0.96	0.18	0.776
D-Dimer (µg/mL)	0.88	0.71	1.20	2.89	0.255
Fibrinogen(mg/dL)	422	251.72	409.57	163.43	0.913
Ferritin (ng/mL)	391.13	530.29	657.00	611.75	0.262
LDH (U/L)	355.36	169.41	236.73	48.99	0.005
CRP (mg/dL)	5.47	7.34	1.84	2.18	0.011
ALT (U/L)	39.08	29.90	111.75	92.100	0.000
AST (U/L)	39.88	13.34	83.00	53.53	0.062
Urea (mg/dL)	31.58	12.17	36.88	10.99	0.031
Creatine (mg/dL)	0.86	0.23	0.75	0.16	0.006
Albumin (g/dL)	4.16	0.63	4.29	2.26	0.790

HGB: Hemoglobin, AST: Aspartate aminotransferase, PT: Prothrombin time, INR: International Normalized Ratio, SD: standard deviation

Table 2 Comparison of GSDMD, Caspase-1, IL-1β and NLRP3 values of case and control groups on hospitalization day and discharge day

	Admission day (n=58)		Discharge day (n=58)		p
	Mean	S.D	Mean	S.D	
GSDMD	4.24	1.33	4.16	1.43	0.85
Caspase-1	5.24	3.86	5.92	5.71	0.96
IL-1β	928.77	878.85	1335.45	1638.85	0.73
NLRP3	99.66	50.17	97.41	49.90	0.34
Comparison of biomarkers of the COVID-19 group with the control group on admission day					
	Control (n=30)		COVID-19 patients' values on admission day (n=28)		p
	Mean	S.D	Mean	S.D	
GSDMD	3.67	0.98	4.24	1.33	0.30
Caspase-1	5.23	3.73	5.24	3.86	0.53
IL-1β	828.06	613.02	928.77	878.85	0.94
NLRP3	86.57	34.48	99.66	50.17	0.79
Comparison of biomarkers of the COVID-19 group with the control group on disharge day					
	Control (n=30)		COVID-19 patients' values on a disharge day (n=28)		p
	Mean	S.D	Mean	S.D	
GSDMD	3.67	0.98	4.16	1.43	0.13
Caspase-1	5.23	3.73	5.92	5.71	0.73
IL-1β	828.06	613.02	1335.45	1638.77	0.98
NLRP3	86.57	34.48	97.41	49.90	0.97

It was compared biomarkers of patients with LDH>250 U/L and those with LDH<=250 U/L at the hospitalization period. As a result of comparisons, it was found that while IL-1β and caspase-1 values were higher at the discharge period, GSDMD and NLRP3 values were lower. However, these differences were not found statistically significant. When the biomarkers of patients with and without the comorbidity were compared at the hospitalization and discharge periods, it was observed that there were no statistically significant differences between GSDMD, caspase-1, IL-1β and NLRP3 values. GSDMD, caspase-1, IL-1β, and NLRP3 values were higher in patients with comorbidities, both at the hospitalization and the discharge (Table 5).

Table 3 GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days

	GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission						
	Group 1 (mildly symptomatic) (n=7)		Group 2 (symptomatic) (radiological involvement in addition to fever and respiratory symptoms) (n=14)		Group 3 (symptomatic (dyspnea, oxygen saturation ≤93% at rest)) (n=7)		p
	Mean	S.D	Mean	S.S	Mean	S.D	
GSDMD	3.61	0.92	4.37	1.08	4.60	1.98	0.53
Caspase-1	4.05	2.16	5.65	3.75	5.61	5.47	0.57
IL-1β	527.31	216.21	1141.90	956	903.96	1073.83	0.10
NLRP3	85.31	42.80	107.37	46.16	98.59	67.05	0.39
	GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of discharge						
	Group 1 (mildly symptomatic) (n=7)		Group 2 (symptomatic) (radiological involvement in addition to fever and respiratory symptoms) (n=14)		Group 3 (symptomatic (dyspnea, oxygen saturation ≤93% at rest)) (n=7)		p
	Mean	S.D	Mean	S.D	Mean	S.D	
GSDMD	4.59	0.88	3.75	1.36	4.55	1.89	0.15
Caspase-1	3.77	1.59	4.95	3.62	10.03	9.40	0.42
IL-1β	594.44	286.55	1185.41	1232.72	2376.52	2598.63	0.61
NLRP3	83.92	36.79	97.44	50.06	110.84	63.22	0.69

Table 4 GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with lymphopenia,leukopenia and neutropenia

GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with lymphopenia (n =28)					
	Admission day		Discharge day		p
	Mean	S.D	Mean	S.D	
GSDMD	4.17	1.51	4.67	1.54	0.64
Caspase-1	6.00	4.66	5.94	5.12	0.51
IL-1β	970.05	854.26	1772.89	2118.20	0.12
NLRP3	109.97	58.79	104.36	59.17	0.35
GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with leukopenia (n=14)					
	Admission day		Discharge day		p
	Mean	S.D	Mean	S.D	
GSDMD	3.91	1.26	4.46	1.23	0.48
Caspase-1	4.37	3.86	4.30	3.78	0.89
IL-1β	876.04	982.73	978.09	1317.66	0.58
NLRP3	87.73	41.70	79.32	35.59	0.16
GSDMD, Caspase-1, IL-1β, NLRP3 values on the day of admission and discharge days in COVID-19 patients with neutropenia (n=7)					
	Admission day		Discharge day		p
	Mean	S.D	Mean	S.D	
GSDMD	3.30	0.28	5.60	0.62	0.11
Caspase-1	2.49	0.88	2.49	0.33	1.00
IL-1β	489.05	292.10	390.02	48.74	1.00
NLRP3	69.31	22.76	63.26	10.17	1.00

Table 5 Comparison of admission and discharge day biomarkers of patients with and without comorbidity

Comparison of the admission day biomarkers of patients with and without comorbidity					
	With Comorbidity (n=15)		Without Comorbidity(n=13)		p
	Mean	S.D	Mean	S.D	
GSDMD	4.27	1.49	4.21	1.18	0.91
Caspaz-1	5.30	4.29	5.17	3.48	0.93
IL-1β	1099.22	1124.30	732.09	430.00	0.79
NLRP3	88.61	41.92	112.41	57.30	0.22
Comparison of the discharge biomarkers of patients with and without comorbidity					
	With Comorbidity (n=15)		Without Comorbidity (n=13)		p
	Mean	S.D	Mean	S.D	
GSDMD	4.44	1.68	3.83	1.03	0.25
Caspase-1	6.83	6.72	4.88	4.29	0.86
IL-1β	1421.72	1532.37	1235.90	1811.90	1.00
NLRP3	89.72	41.95	106.28	58.22	0.44

Discussion

The process of apoptosis was the first type of programmed cell death to be identified, and in most circumstances, caspase-3 and -7 ensure that it is immune-silent. In contrast, the lytic cell death mechanisms of necroptosis and pyroptosis permit the release of potential immunostimulatory chemicals. According to genetic evidence, these cell death pathways can initiate powerful inflammatory responses in vivo, which may contribute to the pathology of numerous inflammatory diseases. In some cases, the bystander DAMPs released after pyroptosis and necroptosis may be less inflammatory than if the cell did not get the cues for suicide. This may reflect cell-type and stimulus-specific pyroptotic and necroptotic signaling scenarios [20-24].

In this study, GSDMD, caspase-1, IL-1β and NLRP3 biomarkers were studied in patients and healthy volunteers with aim of examining the relationship between pyroptosis and COVID-19. In our study, it was detected that there were no statistically significant differences between the COVID-19 and control groups. When the reason of not detecting the significant differences was investigated, it was determined that the deviations between the biomarker values at the hospitalization and the discharge periods were high (high standard deviation) and it is concluded that these results could be caused by the limited population size. Although there were no statistically significant differences, the distribution of some biomarkers, especially IL-1β, between the groups was considered remarkable. Some findings can be summarized as follows: IL-1β level was found to be higher both at the hospitalization and at the discharge periods of COVID-19 patients when compared with the control group. This is thought to be an important indicator of the interaction between COVID-19 and the pyroptosis mechanism. When the patients with high CRP and those without CRP were compared at the hospitalization period, it was observed that all four parameters were higher in those with high CRP values. This suggests that there is a parallelism between the level of inflammation and pyroptosis. At the hospitalization period, the patients with high LDH levels had higher levels of IL-1β. It was observed that IL-1β levels increased as the severity of the COVID-19 increased according to observations obtained at the discharge period. Besides, all four biomarkers were higher in patients with comorbidity both at the hospitalization and at the discharge periods compared to those without comorbidity. This result suggested that other underlying diseases or factors may affect pyroptosis.

Induction of NOD-like receptor (nucleotide-binding oligomerization domain) activation by pathogen or alarmin results in activation of caspase-1. The proteolytic activation of caspase-1 catalyzes the maturation and secretion of proinflammatory cytokines, notably IL-1β and IL-18. NLRP3, the best described among inflammasomes, has been associated with many diseases from autoinflammatory diseases to neurological disorders. NLRP3 also plays a role in antiviral responses and viral diseases [25,26]. There are six members of the gasdermin protein family, and each of them has different functions in human tissues and organs [27,28]. GSDMD is the most common and best studied protein in pyroptosis. GSDMD is cleaved by caspase-1/4/5/11 to release its C and N-terminal fragments. The lipophilic N-terminal disrupts the cell membrane transition balance resulting in K+ outflow to the extracellular space and Na+ entry into the intracellular space by constituting pores in the cell membrane. As a result, the cell swells, the membrane ruptures, and the contents are released out of the cell, causing an intense inflammatory response with pyroptosis [15]. Previous studies have reported that pyroptosis may also cause a strong inflammatory response and clear or reduce cells through division, consistent with symptoms after SARS-CoV-2 infection. There is increasing evidence showing that pyroptosis may play a role in SARS-CoV-2 infection and its pathogenesis [15,29,30]. Xu et al. [18] investigated the relationship between pyroptosis and the severity of COVID-19. In this study, single-cell RNA-seq (scRNAseq) data of 37,607 immune system cells belonging to eight different cell types from four studies were analyzed. As a result of the study, it was determined that the expression of key markers of pyroptosis, such as IL-1β and IL18, was significantly higher in moderate and severe COVID-19 patients than in healthy volunteers. It was observed that caspase-1 was overexpressed in the spleen of hDPP4-Tg (human dipeptidyl peptidase 4 transgenic) mice infected with this virus and elevated levels of IL-1β in the serum. In addition to this, in this study, it was shown that the expression of caspase-1 and IL-1β decreased as a result of blockade of C5a-C5aR1 by an anti-C5aR1 antibody. Based on these data, it has been suggested that MERS-CoV infection induces overactivation of complement, which may contribute to pyroptosis and inflammation, and that C5aR1 may inhibit pyroptosis [31]. Chen et al [32] have stated that SARS-CoV Viroporin 3a activates the NLRP3 inflammasome, triggers IL-1β secretion in bone marrow-derived macrophages, and this suggested cell pyroptosis caused by SARS-CoV.

In our study, it was detected that there were no statistically significant differences between the biomarkers as a result of the mean comparison tests. However, it was observed that the IL-1β levels between groups of the control, the hospitalization, and the discharge were found to be remarkable: in the COVID-19 group, the IL-1β level at the discharge was higher than at the hospitalization; IL-1β levels of the COVID-19 group at both the hospitalization and the discharge periods were higher than the control group. Although statistically significant differences were not detected in our study, the higher IL-1β level in the patient group was considered as an immunological finding supporting the relationship between COVID-19 and pyroptosis. In addition, GSDMD, caspase-1, IL-1β and NLRP3 values were found to be higher in patients having high CRP at the hospitalization period. It was thought that this may be an important indicator of the parallelism between inflammation and pyroptosis. High levels of LDH are a general sign of tissue damage, which is supported by the widespread cell death of monocytes, alveolar epithelial cells, lung, and kidney endothelial cells. These cells are also competent to play a role in inflammasome activation and pyroptosis [33-38]. It has been shown that IL-1 family cytokines, LDH level and high GSDMD expression, which are considered as signs of pyroptosis in plasma, are associated with increasing risk of serious COVID-19 disease [38,39]. Kayagaki et al. [40] showed that IL-1β secretion decreased and release of LDH impaired as a result of blocking the GSDMD pathway in mice. In our study, the high IL-1β level in patients with high LDH level at the hospitalization period supports the relationship between pyroptosis and COVID-19, and in this respect, it is similar to the literature [41-45].

It has been shown that pyroptosis not only has an important role in infectious diseases, cardiovascular diseases, tumors, central nervous system diseases [39-43]. In addition, it has been shown that pyroptosis also plays an important role in the development of spontaneous inflammatory diseases, autoimmune diseases (systemic lupus erythematosus, inflammatory bowel diseases) and various metabolic diseases [46,47]. In our study, GSDMD, caspase-1, IL-1β and NLRP3 values have been found to be higher in patients with comorbidities both at the hospitalization and the discharge periods. This result is thought to be an important indicator that comorbid factors may affect pyroptosis.

Inflammation is induced by the inflammasomes. They have been linked to a number of inflammatory diseases. Our understanding of the processes by which the NLRP3 inflammasome is activated has significantly improved in light of recent evidence. Inflammasome involvement in the onset or progression of diseases with significant effects on public health, such as metabolic pathologies (obesity, type 2 diabetes, atherosclerosis), cardiovascular diseases (ischemic and non-

ischemic heart disease), inflammatory conditions (liver diseases, inflammatory bowel diseases, gut microbiome, rheumatoid arthritis), and neurologic disorders is also strongly supported by growing evidence in animal models and human studies [48]. In this study, the inadequacy of the study population, the heterogeneous distribution of measurements, and not studying of IL-18, another important cytokine released together with IL-1β in the pyroptosis mechanism, are the important limitations of our study.

Conclusion

Despite the decrease in the number of cases, the COVID-19 pandemic continues to maintain its importance. Different studies have been conducted on the etiopathogenesis, and pyroptosis, which is one of the cell death mechanisms, is one of them. In our study, IL-1β, which is one of the cytokines released as a result of cell death in the pyroptosis mechanism, was found to be higher in patients with a diagnosis of COVID-19 both at the hospitalization and the discharge compared to the control group. The high IL-1β level in patients with high LDH levels was another important finding supporting the relationship between pyroptosis and COVID-19. Studies on these pyroptosis-associated markers with larger patient population and different patient groups are needed.

Ethics Committee approval

Harran University, Medicine Faculty Ethics Committee was received the approval (29/11/2021, HRU/21.21.09). All procedures were performed in accordance with the Declaration of Helsinki.

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The effect of therapeutic plasma exchange and intravenous immunoglobulin therapy on biomarkers and 28-day mortality in patients with COVID-19 in intensive care unit

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Abstract

Background: The aim of our study was to determine the effectiveness of the co-administration of therapeutic plasma exchange (TPE) and intravenous immunoglobulin (IVIg) therapy in intensive care patients with COVID-19.

Material and methods: In the propensity-matched study 46 patients were evaluated. The groups were defined as patients who received TPE + IVIg and standard treatment, and patients who received only standard treatment. The primary outcome of the study was determined as a 28-day mortality rate. Secondary outcome measures; were biomarkers of inflammation at admission and treatment days.

Results: In the evaluation of 23 patients in 2 groups, no statistically significant difference was found between demographic data, vital and respiratory status, additional diseases and treatments applied (p>0.05) .There was no difference in 28-day mortality rates between the two groups (p:0.688). CRP, IL-6 and Ferritin Lymphocytes values in the TPE+IVIg group were lower when compared to the control group in the values measured after the treatment (p<0.05). All inflammatory markers applied in the Cox regression model were associated with survival and no association was found.

Conclusion: In the results of this study, in which we applied TPE and IVIg treatment in combination, it was determined that this treatment method did not provide an additional benefit to the standard treatment. More clear information can be obtained by testing treatment applications in different doses and regimens and by randomized controlled studies.

Key words: SARS-CoV 2, COVID-19, intravenous immunoglobulins, therapeutic plasma exchange, intensive care

Introduction

With the definition of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; coronavirus disease 2019 [COVID-19]) as a pandemic infection by the World Health Organization (WHO), many countries have started studies for the diagnosis and treatment of this disease [1]. Coronaviruses (CoV) can cause infections ranging from the common cold to severe disorders such as the Middle East Respiratory Syndrome (MERS) and the Severe Acute Respiratory Syndrome (SARS-CoV) [2,3]. SARS-CoV-2 infection can be transmitted through droplets and mostly asymptomatic and/or self-limited, patients can become critically ill, as manifested by acute respiratory distress syndrome (ARDS), thromboemboli, hyperinflammation and multi-system organ failure (MSOF), which may require intensive care treatment [2-8]. This situation, which occurs due to COVID-19, is related to the cytokine release syndrome, is caused by the late and excessive reaction of the immune system. Since no effective therapy is available, clinicians can use different treatments for this challenging condition in the treatment process. In addition to standard care of treatments (SOC) (Hydroxychloroquine, favipiravir, azithromycin), immunomodulatory treatments,

steroids, intravenous immunoglobulin (IVIg) and extracorporeal treatments are some of them. These treatments, which try to prevent the occurrence of cytokine release syndrome, can be used both as a supportive treatment and to reduce the resulting burden. Among these treatments, therapeutic plasma exchange (TPE) [9] and IVIg can be used in the treatment of different diseases. Apart from removing the abnormal components (immune complexes, toxins, allo/autoantibodies, lipoprotein, monoclonal antibodies, etc.) that play a role in the pathogenesis of diseases, TPE has also been found to have an immunomodulatory effect [10]. IVIg is a liquid preparation containing IgG antibodies with antiviral, bacterial, or other pathogens. IVIg has been identified as a potential mechanism of action, increasing the level of IgG, neutralizing exogenous antigens, and immune regulation. IVIg and TPE have been used in the treatment of bacterial, viral infection, and sepsis in different viral diseases other than COVID-19 [11-13].

Although IVIg and TPE treatments have been used in the treatment of systemic hyperinflammatory response in COVID-19 patients due to this uncontrolled immune response against SARS-CoV-2, the effectiveness of these treatments has not been clearly demonstrated [14-17]. TPE and IVIg combination therapy has been used for immunosuppression [18].

This study hypothesized that TPE and IVIg combination therapy might be effective in preventing systemic hyperinflammatory responses. For this purpose, we investigated the effects on 28-day mortality and biochemical inflammatory markers of patients who received TPE and IVIg combined treatment beside SOC in addition to SOC.

Material and methods

After obtaining ethics committee approval (The decision number is 2011-KAEK-25 2021/07-08) for this trend-oriented retrospective cohort study, the files of patients hospitalized in the intensive care unit (ICU) with the diagnosis of COVID-19 between May 2020 and June 2021 were reviewed.

Severe COVID-19 patients between the ages of 18 and 70 was defined by SARS-CoV-2 positive real-time polymerase chain reaction (RT-PCR test) and requirement for intensive care, based on the presence of the following criteria: (a) respiratory rate >30/min, (b) signs of dyspnea and respiratory distress, (c) SpO2 < 90% and PaO2 < 70 mmHg, despite nasal oxygen support of >10 L/min, or >15 L/min reservoir oxygen mask support (d) PaO2/FiO2 < 300 (mild acute respiratory distress syndrome (ARDS), (e) lactate >2 mmol/L, (f) bilateral infiltrations, multi-lobular involvement or pleural fluid in lung, (g) hypotension (systolic blood pressure <90 mmHg or drop >40 mmHg, mean arterial pressure <65 mmHg), tachycardia >100/min, (h) signs of renal, hepatic, hematologic (thrombocytopenia) or cerebral (confusion) dysfunction (sepsis or septic shock), (i) immunosuppression, (j) troponin elevation and (k) arrhythmia. Exclusion criteria were defined as having a previous allergic reaction to plasma exchange or its ingredients and patients who died 24 hours after administration to ICU. The patients who underwent TPE and IVIg were matched using propensity score matching at a ratio of 1:1 [19]. Matching was performed to equate potential factors affecting patients' mortality for the 2 groups. Tendency scores were calculated using the logistic regression model in which treatment modality was used as a dependent variable. As independent variables, 5 risk factors that were considered to have a direct effect on mortality were determined. Risk factors (1) Age, (2) Gender, (3) Diabetes mellitus, (4) Hypertension, (5) APACHE II score [20]. Trend matching was done using the 1:1 nearest neighbour algorithm. Matches within the limit range of 0.2 standard deviations of the logit of the propensity score were included [21]. All analyzes were restricted to patients compatible with this trend set. After propensity score matching, 2 groups of 23 people were matched. The groups were defined as patients who received TPE+IVIg with SOC, and patients who received only SOC. Initial SOC was planned in accordance with the local pandemic treatment guideline [22], hydroxychloroquine (800 mg loading dose, LD, 400 mg/day maintenance for 5 days) and favipiravir (3200 mg loading dose, 1200 mg/day maintenance for 5 days) were started as first-line therapy. Anticoagulant treatment with Low Molecular Weight Heparine (LMWH) and antithrombotic treatment with acetyl salicylic acid were applied for their admission from the ICU. Considering biochemical markers of inflammation and vital signs, tocilizumab/anakinra, methylprednisolone (1 mg/kg/day), and antibiotherapy were administered as a result of the culture specimen of the patients' body fluids (tracheal aspirate, urine, blood) and the visit made with the infection specialists.

TPE+IVIg treatment were applied to patients who did not find clinical improvement in the treatment protocol described above.

TPE+IVIg therapy; was planned as 5 sessions. It was performed using Fresenius apheresis devices (Fresenius AG, Germany) by subtracting 1.5 times the predicted plasma volume every other day. Body surface area, hematocrit, and gender were used to calculate plasma volumes. During the 4-hour procedure, a 1:1 mixture of fresh frozen plasma (FFP)/human albumin 5% and normal saline was applied as reserve fluid. After the TPE procedure, 10 g of IVIg Octagam® (Octapharma Aglachen, Switzerland) was administered intravenously to each patient with a 6-hour infusion.

The primary outcome of the study was determined as the 28-day mortality rate. Secondary outcome measures were, APACHE II score, observing the changing the biomarkers of inflammation; C-reactive protein (CRP), ferritin, D-Dimer, interleukin (IL) 6 and lymphocyte count (LYM) at admission and on treatment days.

Statistical Method

Descriptive statistics (mean, frequency, percentage, median, min-max, standard deviation,) were used. The Shapiro-Wilk test was used to evaluate the distribution model. Wilcoxson test or Mann-Whitney U test was used for comparison between groups and in-group measurement times. A main effect logistic regression model was used to examine the effect of treatment on overall survival. The effect of biochemical values on survival times was evaluated using Cox regression models. The Kaplan-Meier test was used for survival analysis and log-rank was used to compare the difference between the two groups. A p-value less than 0.05 was determined as the level of significance.

Results

In this study, the data of 46 patients were subjected to statistical analysis. In the evaluation of 23 patients in two groups, no statistically significant difference was found between demographic data, vital and respiratory status, additional diseases and treatments applied (p>0.05) (Table 1). There was no difference in 28-day mortality rates between the two groups. Kaplan–Meier survival distributions in the TPE+IVIg and control groups patients (log-rank test, P=0.688; Cox regression model, Hazard Ratio=0.81 confidence interval (95% CI 0.335-2.029, P=0.62) (Figure 1). There was no significant difference

Table 1 Patient characteristics

	TPE+IVIg *n =23*	Control *n =23*	*p*
Sex (M/F)	8/15 (65/35)	10/13 (74/26)	0.621
Age (years)	45.3 ± 18.2	48.3 ± 12.2	0.657
BMI (kg/m^2)	25.8 ± 5.3	24.9 ± 4.1	0.633
Vital and respiratory status			
APACHE II score	24.3 ± 5.3	25 ± 7.9	0.681
Respiratory rate (/min)	36 ± 9	35 ± 7	0.678
PaO2/FiO2 ratio	121 (90–165)	128 (90–195)	0.137
High-flow nasal cannula	17	16	0.844
Mechanical ventilation	6	7	0.708
Systolic blood pressure (mmHg)	109.8 (116-84)	108.88 (104-84)	0,742
Diastolic blood pressure (mmHg)	68.72 (94-34)	67 (99-44)	0,436
Additional diseases			
Hypertension	11 (47.0%)	12 (52.1%)	0.893
Diabetes	8 (34.7%)	10 (43.4%)	0.729
Cardiac disease	5 (21.7%)	6 (26%)	0.722
Pulmonary disease	3 (13%)	1 (4.3%)	0.347
Treatments			
Favipiravir	23 (100%)	23 (100%)	N/A
Hydroxychloroquine	21 (91.3%)	20 (86.9%)	0.981
Azithromycin	3 (13%)	4 (17.3%)	0.943
Tocilizumab	10 (43.4%)	12 (52.1%)	0.637
Anakinra	8 (34.7%)	6 (26%)	0.577
LMWH	23 (100%)	23 (100%)	N/A
Corticosteroids	21 (91.3%)	22 (95.6%)	0.781
ICU day	16.5(7-28)	18.7(9-36)	0.559
Mortality on Day 28 [n (%)]	13 (56.5%)	14(60%)	0.767

APACHE II, Acute Physiology and Chronic Health Evaluation II; BMI, Body mass index; FIO2, Fraction of inspired oxygen; ICU, Intensive care unit; PaO2, Arterial partial pressure of oxygen;
Values are means ± SD (n) or N (%), except ,median (interquartile range) due to non-normal distribution. p-values <0.05 in bold.

Table 2 Change in Biomarkers before and after treatment

	TPE+IVIg * *n =23*	Control* *n =23*	*p**
CRP mg/L			
Baseline	129 (35–302)	122 (31–189)	0.648
Post treatment	57 (19–100)	82 (14–216)	**0.044**
p	**0.000**	0.246	
IL-6 µg/L			
Baseline	104 (16–194)	108 (15–176)	0.723
Post-treatment	21 (1–76)	55 (7–477)	**0.001**
p	**0.000**	0.84	
Ferritin mg/L			
Baseline	1061 (336–2000)	1141 (31–1890)	0.677
Post-treatment	590 (285–889)	924 (285–2000)	**0.013**
p	**0.001**	0.078	
D-dimer mg/L			
Baseline	2.1 (0.46–8.8)	3.3 (0.92–8.9)	0.703
Post-treatment	1.94 (1.1–4.6)	3.2 (2.2–6)	0.530
p	0.940	0.573	
Lymphocytes 10^9/L			
Baseline	0.4 (0.18–0.63)	0.31 (0.17–0.45)	0.364
Post-treatment	0.41 (0.17–1.32) (n =22)	0.41 (0.16–0.63)	0.276
p	**0.002**	**0.033**	

Post-treatment: 8 days after initiation of treatment
*median (interquartile range)

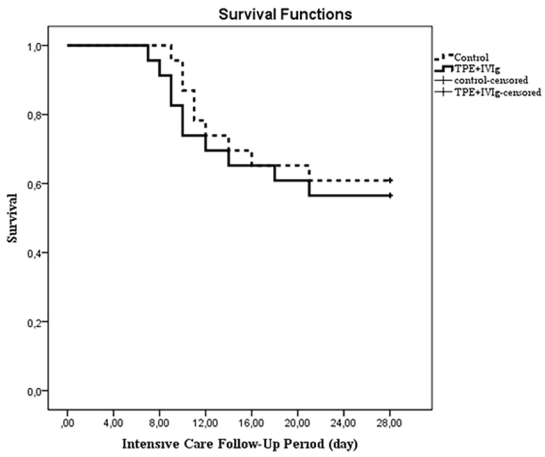

Figure 1 - Kaplan–Meier survival distributions in the TPE+IVIg and control groups patients

Table 3 Cox proportional hazards model for biochemical markers for 28-day mortality in patients (n=46)

	HR p	95% CI	p
IL-6 µg/L	1.003	0.993-1.012	0.580
Ferritin mg/L	0.999	0.998-1.008	0.237
CRP mg/L	1.002	0.997-1.008	0.383
D-dimer mg/L	0.982	0.995-1.009	0.175
Lymphocytes 10^9/L	0.890	0.156-8.488	0.890

CRP, C-reactive protein; PCT, Procalcitonin;IL-6, Interleukin 6; significant differences between groups in bold. HR:Hazard Ratio

between the initial values in the measurements of CRP, IL-6, D-Dimer, Lymphocytes and Ferritin, which are used to monitor the follow-up and treatment response ($p>0.05$) (Table 2). When the values before and after the treatment were evaluated, the CRP, IL-6, D-Dimer and Ferritin values of the patients in the TPE+IVIg group were found to be low after the treatment, while in the control group, only Lymphocytes values were lower than the initial values after the follow-up ($p<0.05$) (Table 2). When the values measured after treatment were compared, TPE+IVIg group had lower CRP, IL-6 and Ferritin values ($p<0.05$) (Table 2). CRP, IL-6 and Ferritin values in the TPE+IVIg group were lower when compared to the control group in the values measured after the treatment ($p<0.05$) (Table 2). All inflammatory markers applied in the Cox regression model were associated with survival and no association was found (Table 3).

Discussion

In the results of this study, no statistically significant difference was found in the 28-day mortality of the patients in the control group treated with SOC in combination with TPE + IVIg combined treatment with SOC. The mortality of the patients was associated with the cytokine storm and acute respiratory failure caused by COVID-19. Although IL6, Ferritin, and CRP values, which are biochemical markers showing inflammation, were lower in the follow-ups of patients treated with TPE+IVIg, they were not associated with mortality.

TPE was started to be used for the first time in the early 1900s and started to be used in the treatment of different diseases in 2013 under the name of therapeutic plasma exchange. Theoretically, it is aimed to reduce the immune load in the body by separating the plasma from the blood and applying replacement fluid instead. Its immunomodulatory effect has been demonstrated in different studies [10]. It has been determined that this immunomodulatory effect occurs in the form of stimulating proliferation of B cells and plasma cells, removal of immune complexes with macrophage/monocyte function, replacement of deficient plasma components such as ADAMTS13, removal of cytokines, changes in lymphocyte counts, and correction of the modified T helper cell type 1/2 (Th1/Th2) ratio that supports Th1 dominance [10]. The American Society for Apheresis (ASFA) periodically updates and publishes guidelines on which diseases TPE can be beneficial and can be used. For sepsis and macrophage activation syndrome, it has been reported that TPE can be used in certain patients whose Category 3 grade 3c efficacy cannot be determined in this guideline [10]. There are few studies at a similar level in the literature. In their retrospective, observational study and review results, Ketih et al. showed an improvement in 28-day survival with adjunctive TPE compared to standard care alone in adult patients with septic shock and multi-organ failure [13]. It has been reported that hemodynamics, organ dysfunction, and fluid balance can be corrected with additional TPE, and survival times can be increased [13].

A limited number of studies in the literature provide information about the effectiveness of TPE application in the treatment of COVID-19. While there are studies indicating that TPE is effective in treatment and survival, some studies found that it does not affect mortality. In the study in which the results of 11 patients who underwent TPE were shared, it was stated that mortality and extubation time decreased with the application of TPE compared to the patients used as the control group [23]. In addition, they found a decrease in SOFA scores, IL-6, CRP, D-dimer, and ferritin levels after TPE application [23]. Another study, sharing the results of 15 COVID-19 patients after TPE treatment additionally used convalescent plasma in 4 patients. In this study, in which TPE treatment was determined to be effective on mortality, they determined a decrease in inflammatory markers [20]. Patidar et al. shared an opinion that TPE can be used as a treatment option in the guideline for its use in infectious diseases and COVID-19. They stated that the weak side of the guideline is the absence of RCT [24].

On the other hand, in a randomized controlled study in the literature, Faqihi et al. evaluated 83 patients and reported that TPE added to standard treatment in life-threatening COVID-19 patients provided clinical improvement compared to standard treatment alone, but did not significantly affect 35-day mortality [15]. Low baseline PaO2/FiO2 ratio, ADAMTS-13 activity, higher SOFA score, increased D-dimer levels and IL-6 were determined as predictors of mortality [15]. While the number of TPE treatments applied in the examined studies varied between 4-5, it varied according to the availability of fluids used for replacement and the conditions of the country. The fact that it is a device-dependent treatment and the need for additional personnel can reduce usability in pandemic conditions. On the other hand, it can be said as an advantage that the IVIg treatment option can be applied more easily. Therefore, the literature data also includes more studies. Shao et al., from 2 different studies designed retrospectively from these studies, reported that 28-day mortality and inflammation could be reduced in patients treated with IVIg and SOC in their cohorts. In the subgroup analysis, they found a better response in patients who started early treatment (before 7 days) with a high dose of more than 15 g/day [17]. In other retrospective study results, the dose of IVIg was determined as 30 g/day at 5% concentration for 5 days. In this study, in which they found a significant decrease in survival times compared to the patient group that they applied standard treatment, they emphasized that the decrease

in CRP values was significant in the follow-up. On the other hand, they found that the decrease in IL-6 and the increase in D-dimer were not significant [17]. In the RCT found in the literature, it was stated that the use of 400 mg/kg IVIg for 3 days and hydroxychloroquine, lopinavir/ritonavir as an additional treatment did not have an effect on mortality and did not affect the radiological changes. In addition, it was emphasized that early IVIg treatment may shorten the length of hospitalization [26]. In their study, Bongomin et al. reached similar results and stated that it did not provide additional benefit in non-severe COVID-19 and that IVIg could help treatment in combination with other drugs such as corticosteroids or antibiotics [27].

When the literature information is examined, it does not seem possible to reach definitive data on the effectiveness of TPE and IVIg treatment options alone on the hyperinflammatory state caused by SARS-CoV-2. Considering the global pandemic, the fact that physicians are courageous about alternative treatments should be taken into consideration. Dosing times and procedures differ between the two treatment options, and the impact of this on study results is debatable. The differences in the medical treatment applied by countries during the pandemic limit the comparison of studies. TPE+IVIg combination treatment method used in our study did not provide more survival when compared to the control group patients. On the other hand, when compared to the studies in the literature, the late treatment initiation and the severe clinical condition of selected patient groups may have affected the results.

Limitations

Although the patients in the study were matched, the retrospective design is the main limitation of this study. In addition, due to small sample size of our study and the differences in the medical treatments applied for immune modulation during the treatment of both groups of patients stands out as another limitation.

Conclusion

In the results of this study, in which we applied TPE + IVIg treatment in combination, it was determined that this treatment method did not provide an additional benefit to the standard treatment. More clear information can be obtained by testing treatment applications in different doses and regimens and by randomized controlled studies.

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Assessment of risk factors for thrombosis in ICU patients with COVID-19

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Abstract

Introduction: High incidence of thrombotic events has been reported in hospitalized patients with COVID-19. Less than 50% of pulmonary embolisms (PE) are associated with signs of deep vein thrombosis (DVT) of the lower extremities.

Objective: To assess the risk factors of deep vein thrombosis (DVT) in intensive care patients with COVID-19 by comparing the clinical features of patients in groups with thrombosis, venous stasis and without deep vein thrombosis.

Material and methods: A prospective cross-sectional study was conducted that included all consecutive adult patients with laboratory-confirmed COVID-19 admitted to the intensive care unit. We investigated chronic comorbid conditions in patients, including arterial hypertension, diabetes mellitus, obesity, chronic kidney failure (CRF), chronic obstructive pulmonary disease (COPD), chronic heart failure (CHF), and cancer which may be a risk factor for thrombosis.

Results: A total of 465 patients were included in the study. Comorbidities were present in 435 of 465 patients (93.55%). Doppler ultrasound (DUS) confirmed deep vein thrombosis in 60 patients (13.8%), which was associated with older age (71.12±13.98 vs. (79.57%), chronic heart failure - 196 (42.15%), obesity - 161 (34.62%), diabetes mellitus - 144 (30.97%), chronic renal failure (CRF) -58 (12.47%) and oncological diseases - 25 (5.38%). Hypertension (p=0.02), diabetes mellitus (p=0.041) and obesity (p=0.01) were significant risk factors for DVT. D-dimer was a statistically significant predictor of DVT formation (p<0.001), an increase in D-dimer per unit increased the risk of DVT by 14%.

Conclusion: The study identified risk factors for deep vein thrombosis in intensive care patients with COVID-19. These include: age, high levels of D-dimer, and comorbidities such as hypertension, obesity, and diabetes mellitus.

Key words: COVID-19, deep vein thrombosis (DVT), thromboembolism, ultrasound diagnostics

Introduction

After the first case of coronavirus (COVID-19) in Wuhan, China in late December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread to more than 200 countries in about 3 months. On March 11, 2020, the World Health Organization (WHO) declared the outbreak a pandemic [1-3].

A high incidence of thrombotic events has been reported in hospitalized patients with COVID-19 [4–17]. Most patients suffer from venous thromboembolic events, with pulmonary embolism (PE) playing a major role and this has an impact on the outcome of the disease [10-17].

One hypothesis is that isolated pulmonary microcirculatory thrombosis of the lung may be the cause of severe atypical cases of acute respiratory distress syndrome (ARDS) of COVID-19 pneumonia. However, PE may occur in patients with COVID-19, especially if clinical suspicion is confirmed by instrumental diagnosis of deep vein thrombosis (DVT) of the lower extremities. Several studies have specifically examined the incidence of DVT in patients with COVID-19 pneumonia or the role of blood tests such as D-dimer in detecting DVT [5–9].

Several mechanisms may contribute to the procoagulant state in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). First, it has been demonstrated that an inflammatory condition occurs during COVID-19 that causes endothelial cell dysfunction and leads to increased thrombin production and impaired fibrinolysis. Second, hypoxia can stimulate thrombosis by increasing blood viscosity and inducing signaling pathways dependent on transcription factors [18, 19].

Purpose of the study: To identify significant risk factors for deep vein thrombosis (DVT) in intensive care patients with COVID-19.

Material and methods

We conducted a prospective cross-sectional study that included all adult patients with a laboratory-confirmed diagnosis of COVID-19 admitted to 3 intensive care units of 3 hospitals in Astana with previously undiagnosed DVT or pulmonary embolism (PE). A total of 465 patients were included in the study who developed PE followed by death.

The inclusion criteria for study subjects were patients 18-98 years of age with deep vein thrombosis in COVID-19, of any nationality, who signed an informed consent to participate in this study or was signed from relatives, since 65% of patients were in critical condition (who were on ventilator), with several of the signs and symptoms suggestive of DVT in COVID-19 (swelling of the lower extremities, bluish skin tone, and pain with movement of the lower extremities), with severe and unstable comorbid somatic pathology (diabetes mellitus (DM) , arterial hypertension (AH), obesity, chronic renal failure (CRF), chronic heart failure (CHF), chronic obstructive pulmonary disease (COPD) and cancer. The study also included patients with COVID-19, with elevated plasma levels of D-dimer or changes in the coagulogram (fibrinogen, factor VIII).

Exclusion criteria were patients under 18 years of age, pregnant women, patients after trauma, after surgery, patients with prolonged immobilization prior to COVID-19 infection, patients who had arteriovenous fistula as well as all other patients without COVID-19 with deep vein thrombosis. The inclusion period was 04/22/2020-11/26/2020. Our study was approved by the Local Ethics Committee (Figure 1).

Oropharyngeal swabs were taken upon admission to the hospital in accordance with the protocol of the Republic of Kazakhstan. Condition assessment and disease monitoring were performed along with serological testing for SARSCoV-2 PCR RNA or detection of antibodies against SARSCoV-2, as well as on the basis of the results of a complete blood count, coagulogram, D-dimer, fibrinogen, saturation determination.

Upon admission to the emergency department, all patients underwent computed tomography (CT) of the chest, which revealed bilateral lung disease (ground-glass syndrome, interstitial lung disease) corresponding to viral pneumonia. The degree of lung involvement was classified as ≤ 30%, 31–50%, and ≥ 50% of the total lung area. Lung CT angiography was performed in all patients with high clinical suspicion of pulmonary embolism/deep vein thrombosis (PE/DVT).

166 patients were examined using duplex ultrasound (DUS) in B-mode and, if necessary, with color Doppler blood flow mapping. The studies were carried out on a LOGIQ - 6 and VOLUSON 730 EXPERT device (GE Healthcare, USA) using linear sensors operating in the frequency range of 5–10 MHz. The state of the deep veins of the lower extremities was assessed by ultrasound diagnostic doctors with more than 5 years of experience. The deep veins included in the study were the femoral, popliteal, and distal veins (posterior tibial, peroneal, gastrocnemius, and soleus veins) of the lower extremity. The examined superficial veins were also the great and small saphenous veins of the lower extremity. Lack of compressibility or direct identification in the lumen of a thrombus was used as criteria for the diagnosis of thrombosis. Compression was performed in the transverse plane to avoid slipping of the probe from the vessel wall along the longitudinal axis, which can lead to false negative results. In addition, testing was considered or possibly repeated if DVT was clinically suspected.

During the examination, we took into account the presence of chronic concomitant diseases in the studied patients, such as arterial hypertension, diabetes mellitus, obesity, chronic renal failure (CRF), chronic obstructive pulmonary disease (COPD), chronic heart failure (CHF) and oncological diseases.

For categorical variables, a chi-square test and Fisher's exact test (for subgroups of 5 or fewer people) were used. For quantitative variables, analysis of variance (ANOVA) and Pearson and Spearman correlations were used. For multiple variables, ordered logistic regression models were built, with likelihood ratio tests performed to compare the models. Two-tailed p-significances <0.05 were presented as statistically significant. All calculations were performed using STATA MP 17.0 software (StataCorp LLC).

Results

The mean age (+-SD) of all examined patients was 70.58±11.84 years (range 25 to 98 years), these were all adult patients admitted to the intensive care unit during the pandemic, among which only 2 patients were over the age of 90 years. The sex ratio was 272 (58.49%) : 193 (41.51%) (male: female). The average body mass index was 29.7 kg/m2. Of 465 (44.3%) patients, 206 had a BMI greater than or equal to 30. Of these, 34 (16.5%) patients had DVT.

We selected 166 patients who underwent ultrasonography of the deep veins of the lower extremities and were divided into 3 groups (Table 1).

Table 1 Association of quantitative variables with DVT (mean ± standard deviation (min; max))

	Patient groups			Total	p-value
	No DVT	Venous stasis	DVT		
Age	65.20±11.16 (25;100)	67.23±13.06 (31;91)	71.12±13.98 (36;94)	70.58±11.84 (25;100)	0.006
BMI kg/m2	29.22±6.46 (12.30;60.55)	30.24±6.85 (12.80;49.13)	32.57±10.92 (15.10;70.86)	29.78±7.30 (12.30;70.86)	0.358
D-dimer (µg/ml)	3.24±3.88 (0.1;38.49)	2.96±2.60 (0.12;16)	6.46±3.66 (1;18.5)	3.62±3.87 (0.1;38.49)	0.001
Fibrinogen (g/l)	5.13±1.75 (0.39;15.80)	4.83±1.41 (2.55;9.60)	5.36±2.38 (2.80;20.20)	5.12±1.80 (0.39;20.20)	0.001
SpO2 (%)	78.74±12.71 (34;98)	77.39±11.59 (34;93)	68.22±12.05 (30;94)	77.22±12.95 (30;98)	<0.001
CT (%)	46.35±21.03 (4;98)	47.39±18.79 (13;90)	63.98±16.72 (25;95)	48.75±21.07 (4;98)	0.003

BMI - body mass index, SpO2 saturation, CT - computed tomography

According to the results of the obtained ultrasound data, all patients were divided into 3 groups: with DVT - 60 patients (13.8%) (mean age 71.12±13.98 years), without DVT – 349 patients (74.2%) (67.20±11.16 years), with venostasis – 56 patients (12%) (67.23±13.06) (Figure 1).

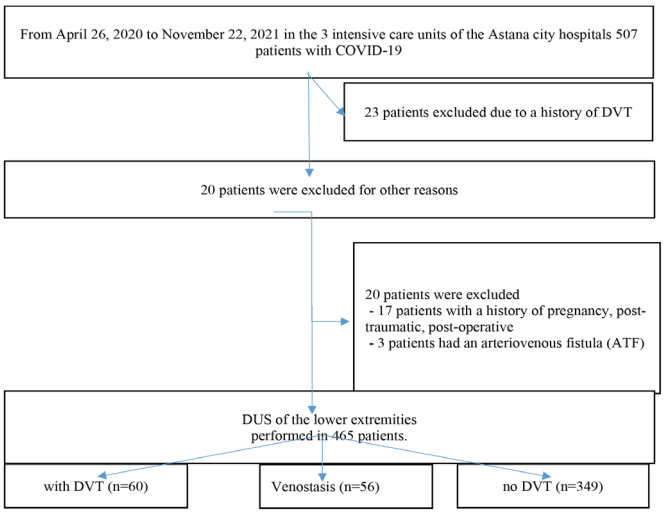

Figure 1 - Selection of patients for study. DVT - deep vein thrombosis; DUS- Doppler ultrasonography

It should be noted that in the group with DVT, the mean age of patients was significantly older than in the group without DVT (p<0.006). When comparing the body mass index in the study groups, it turned out that in patients in the group with DVT and in the group with venostasis, BMI was more than 30 kg/m2 than in the group without DVT (32.57±10.92, 30.24±6.85 versus 29.22±6.46) (Table 1).

In the majority of cases, DVT was detected in the tibial segment 26 (43.33%), in 18 (30%) patients it was diagnosed in the popliteal veins and in 14 (23.33%) cases in the femoral segment.

Coagulation tests showed that D-dimer levels were significantly higher in the DVT group compared to the non-DVT group (6.46±3.66 vs. 3.24±3.88 µg/mL, p<0.001). We obtained a similar difference when assessing the level of fibrinogen in the group with DVT and in the group without DVT (5.36±2.38 versus 4.83±1.41 g/l, p<0.002).

Fibrinogen (5.1 g/L; range 4.5–7.2) and D-dimer (3.6 µg/L; range 0.5<) levels were high. The level of D-dimer ranged from 0.1 µg/ml to 38.49 µg/ml with an average value of 3.62±3.87 µg/ml (Table 1). An increase in D-dimer (≥ 0.50 mg/l) was observed in 90.75% (422/465) of patients.

In all patients, CT scanning of the chest revealed bilateral ground-glass changes and consolidation of the lung tissue. In the

Table 2 Association of qualitative variables with DVT

	Patient group			Total	p-value
	No DVT	Venous stasis	DVT		
Gender					0.809
Female	202 (57.88%)	35 (62.50%)	35 (58.33%)	272 (58.49%)	
Male	147 (42.12%)	21 (37.54%)	25 (41.67%)	193 (41.51%)	
PCR					0.338*
Yes	343 (98.28%)	54 (96.43%)	58 (96.67%)	455 (97.85%)	
No	6 (1.72%)	2 (3.57%)	2 (3.33%)	10 (2.15%)	
Vaccination					0.691
Yes	30 (8.6%)	6 (10.71%)	7 (11.67%)	43 (9.25%)	
No	319 (91.4%)	50 (89.29%)	53 (88.33%)	422 (90.75%)	
X-ray					0.813*
Pneumonia	333 (95.42%)	53 (94.64%)	57 (95%)	443 (95.27%)	
Pleural effusion	16 (4.58%)	3 (5.36%)	3 (5%)	22 (4.73%)	
Hypertension					0.289
Yes	278 (79.66%)	41 (73.21%)	51 (85%)	370 (79.57%)	
No	71 (20.34%)	15 (26.79%)	9 (15%)	95 (20.43%)	
Diabetes mellulitis					0.004
Yes	103 (29.51%)	12 (21.43%)	29 (48.33%)	144 (30.97%)	
Obesity					0.006
Yes	108 (30.95%)	22 (39.29%)	31 (51.67%)	161 (34.62%)	
No	241 (69.05%)	34 (60.71%)	29 (48.33%)	304 (65.38%)	
CHF					0.424
Yes	153 (43.84%)	20 (35.71%)	23 (38.33%)	196 (42.15%)	
No	196 (56.16%)	36 (64.29%)	37 (61.67%)	269 (57.85%)	
COPD					0.581
Yes	36 (10.32%)	8 (14.29%)	8 (13.33%)	52 (11.18%)	
No	313 (89.68%)	48 (85.71%)	52 (86.67%)	413 (88.82%)	
CHF					0.011*
Yes	51 (14.61%)	1 (1.79%)	6 (10%)	58 (12.47%)	
No	298 (85.39%)	55 (98.21%)	54 (90%)	407 (87.53%)	
Cancer					0.146
Yes	15 (4.3%)	4 (7.14%)	6 (10%)	25 (5.38%)	
No	334 (95.7%)	52 (92.86%)	54 (90%)	440 (94.62%)	

Note: CHF-Chronic heart failure, COPD- Chronic obstructive pulmonary disease, CRF- Chronic renal failure

Table 3 Logistic regression. Outcome selected as 2 - DVT, 1 - venostasis, 0 - no DVT

	OR (95% CI)	p-value
Age	0.99 (0.97; 1.01)	0.176
D-dimer	1.15 (1.08; 1.22)	<0.001
Gender		0.935
Female	1	
Male	1.02 (0.64; 1.62)	
Hypertension		0.02
Yes	1	
No	0.70 (0.39; 1.25)	
Diabetes mellulitis		0.041
Yes	1.64 (1.02; 2.62)	
No	1	
Obesity		0.01
Yes	1.80 (1.15; 2.82)	
No	1	
CHF		0.382
Yes	0.81 (0.51; 1.30)	
No	1	
COPD		0.108
Yes	1.70 (0.89; 3.26)	
No	1	
CRF		0.028
Yes	0.39 (0.17; 0.90)	
No	1	
Cancer		0.081
Yes	2.15 (0.91; 5.07)	
No	1.00 (;)	

Note: CHF-Chronic heart failure, COPD- Chronic obstructive pulmonary disease, CRF- Chronic renal failure

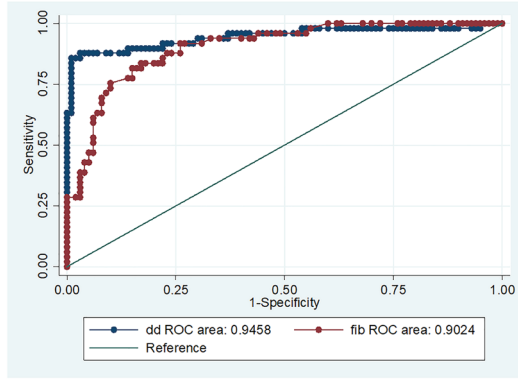

Figure 2 - Dependence of D-dimer and the degree of lung damage on CT

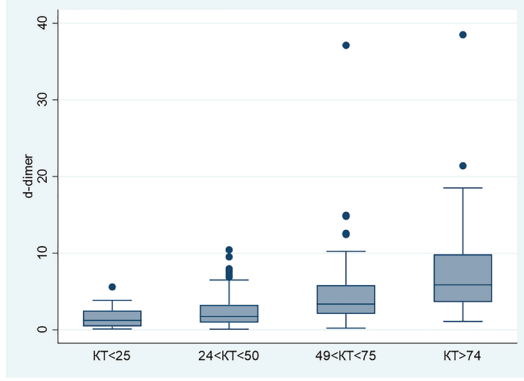

Figure 3 - ROC analysis for statistically significant variable (D-dimer, Fibrinogen

Table 4 Studies to identify venous thromboembolic complications. 2021 meta-analysis results

Authors	Country	Study design	Number of patients	Male	Age	Severity	Anti-coagulants	End points	CT-angio-graphy	PE (%)	DVT (%)
Grillet	France	single center	100	70	66±13	Severe	Unknown	PE	100	23	12.6
Leonard-Lorant	France	single center	106	70	62.5±14.3	Mixed	Yes	PE	100	30.2	23.0
Gervaise	France	single center	72	54	62.3±17.8	Mixed	No	PE	100	18.1	14
Klok	Netherlands	prospective multicenter	184	139	64±12	ICU	Yes	PE, DVT	unknown	35.3	0.5
Al-Samkari	USA	multicenter	400	228	62	Mixed	Yes	PE, DVT	unknown	2.5	2.5
Zhang	China	single center	143	74	63±14	Mixed	Yes	PE, DVT	2.1	0.7	46.2
Ren	China	multicenter	48	26	70 (62-80)	ICU	Yes	DVT	unknown		85.4
Llitjos	France	multicenter	26	20	68 (51.5-74.5)	ICU	Yes	PE, DVT	unknown	23.1	50
Helms	France	prospective multicenter	150	122	63	ICU	Yes	PE, DVT	66.7	16.7	2
Demelo-Rodriguez	Spain	prospective singlecenter	156	102	68.1±14.5	Mixed	Yes	DVT	unknown		14.7
Middledorp	Netherlands	single center	198	130	61±14	Mixed	Yes	PE, DVT	unknown	6.6	12.6
Cui	China	single center	81	37	59.9±14.1	ICU	No	DVT	unknown	-	13.7
Poissy	France	single center	107	78	60.8±14	ICU	Yes	PE, DVT	31.8	20.6	4.7
Bompard	France	multicenter	135	94	64 (65-76)	Mixed	Yes	PE	100	23.7	-
Cattaneo	Italy	singlecenter	64	35	70 (58-77.5)	Mixed	Yes	PE	unknown	-	0
Maatman	USA	multicenter	109	62	61 ±16	ICU	Yes	PE, DVT	unknown	4.6	22
Hekimian	France	singlecenter	51	38	51.9±11	Mixed	Yes	PE	64.7	15.7	-
Artifoni	France	multicenter	71	43	64 (46-75)	ICU	Yes	PE, DVT	45.1	9.9	21.1
Grandmaison	Switzerland	singlecenter	29	18	64.5 ±10	ICU	Yes	PE, DVT	41.4	6.9	38.6
Fraisse	France	singlecenter	92	73	61 (55-70)	ICU	Yes	PE, DVT	29.3	28.3	13
Lodigiani	Italy	singlecenter	362	264	66 (55-85)	Mixed	Yes	PE, DVT	8.3	2.8	1.7
Longchamp	Switzerland	singlecenter	25	16	68±11	ICU	Yes	PE, DVT	36	24	24
Poyiadji	USA	multicenter	328	186	62±15	Mixed	Yes	PE	100	22	-
Fang	Great Britain	singlecenter	93	60	62 (56-69)	Mixed	Yes	PE	100	44.1	-
Valle	Italy	multicenter	114	84	61 (51.2-66)	Mixed	Yes	PE	100	57	18
Manjunath	USA	singlecenter	23	15	61.7	ICU	Yes	PE	43.5	26.1	16,3
Kerbikov	Russia	singlecenter	75	36	63.4	Severe	Yes	PE	unknown	-	20
Ozerman	Kazakhstan	prospective singlecenter	465	193	70.58±11.84	ICU	Yes	PE, DVT	unknown	34.4	13.8

DVT group, the percentage of lung involvement was higher than in the other two study groups (63.98±16.72 vs. 46.35±21.03, 47.39±18.79). Figure 2 shows the D-dimer values in accordance with the degree of lung damage detected on CT. D-dimer values in patients with CT-4 COVID-19 were 4 times higher than in patients with CT1 COVID-19 (median 7.12±5.65 μg/mL versus 1.53±1.20 μg/mL, respectively).

According to the ROC analysis, values for D-dimer >=2.33 μg/ml in the studied patients with a sensitivity of 87.76% and a specificity of 97.00% affect the risk of thrombosis. We also found that 55/60 (91.7%) patients with DVT had D-dimer levels >2.33 μg/mL, while 5/60 patients (8.3%) with DVT had D-dimer levels <2.33 μg /ml Fibrinogen values >=4.64 g/L indicate thrombus formation with a sensitivity of 83.67% and a specificity of 83.00% (Figure 3). Of the two parameters studied, D-dimer (AUC area = 0.9458) is more accurate than fibrinogen (AUC area = 0.9024).

Comorbidities 435 of 465 patients (93.55%) had at least one comorbidity. The most common were arterial hypertension in 370 patients (79.57%), chronic heart failure in 196 patients (42.15%), obesity in 161 patients (34.62%), diabetes mellitus in 144 patients (30.97%), chronic renal failure (CRF) in 58 patients (12.47%) and oncological diseases in 25 patients (5.38%) (Table 2).

Logistic regression analysis showed that the level of D-dimer may indicate the risk of developing DVT. According to our study, D-dimer was a statistically significant predictor of DVT formation (p<0.001), an increase in its value by one unit increases the risk of DVT formation by 14%. Diabetes mellitus (p=0.041) and obesity (p=0.01) were also significant risk factors for DVT. Diabetes mellitus is associated with a 64% increased risk of DVT (Table 3). Obesity increases the chance of DVT by 80%. As for chronic renal failure, in our case, patients with CRF were less likely (by 61%) to suffer from DVT, compared with those who did not have CRF (p=0.028).

Discussion

Due to the high mortality of patients with COVID-19, the first study we conducted in Kazakhstan revealed the main risk factors for the development of thromboembolic events. In this study, we identified the incidence, prevalence, and risk factors for DVT in critically ill patients with COVID-19. DVT is a complication and one of the causes of death in intensive care patients with COVID-19. According to the results of our study, DVT 13.8% occurred as often as in neighboring countries such as Russia (20%) and others (Table 4). Elderly patients who either have comorbidities (obesity, arterial hypertension, diabetes mellitus, chronic heart failure (CHF), chronic obstructive pulmonary disease (COPD), chronic renal failure (CRF)) or are in intensive care (mortality 21.9% in patients over 80 years of age) are at particularly high risk of developing thromboembolic complications [20-21]. In our study, DVT was associated with older age compared with patients without DVT (71.12±13.98 vs. 67.20±11.16, p<0.006).

According to Zhou, F. and al. comorbidities in DVT may increase the risk of pulmonary embolism [22], which is consistent with our results where almost all patients (435 of 465 patients) (93.55%) had at least one comorbidity. This proves that comorbidities such as arterial hypertension, diabetes mellitus, CHF, obesity, which have a high frequency in our study, may also play a role in the development of DVT.

Several researchers have drawn attention to the potential over-prevalence of arterial hypertension among patients with COVID-19 [22-25]. Moreover, hypertension appears to be strongly associated with age, becoming one of the strongest predictors of death associated with COVID-19 [26]. In particular, observational and retrospective studies conducted near the Wuhan area have actually shown that hypertension is the most common comorbidity seen in COVID-19 patients, ranging from 15% to over 30% [25,26]. In our study, in 370 patients (79.57%) with COVID-19, arterial hypertension was the most common comorbidity. Although it was observed in 51 patients (85%) in the group with DVT, however, it was not a significant risk factor for the development of DVT in our case. However, we believe that other comorbidities, such as diabetes mellitus and obesity, may increase the risk of venous thromboembolic complications against the background of arterial hypertension.

Diabetes mellitus is a common comorbidity and causes a worse prognosis in patients with COVID-19 [31-33]. In patients with COVID-19, the incidence of diabetes is twice as high in intensive care units with severely ill patients [27–30]. Indeed, in our study in the group with DVT, 51 patients (85%) had diabetes mellitus and it was a significant risk factor for the occurrence of DVT. In our study, logistic regression proved that diabetes mellitus (p=0.041) was associated with a 64% increased risk of DVT.

Patients with CHF are also at high risk of mortality from COVID-19. According to foreign researchers, heart failure changes the blood coagulation system, which leads to edema of the lower extremities [31-32]. Based on the results of the study, CHF was observed in second place in terms of frequency of occurrence among concomitant diseases, after arterial hypertension (196/465 (42.15%) patients). Moreover, in the group without DVT, CHF was more common than in the group with DVT, and therefore, according to our data, it is not a significant risk factor for the occurrence of DVT (p=0.424).

According to the literature, it is known that if a person's BMI is more than 30 cm2 or a person is obese, then he has an increased risk of severe disease or mortality from SARSCoV-2 infection, and is also at risk of thrombosis [33]. We found a similar relationship in our study: in the group with DVT, 31/60 patients (51.67%) had obesity and was a significant risk factor for the occurrence of DVT. This means that more than half of the patients with thrombosis were obese, while in the group without DVT, obesity was observed in only a third of patients. (p=0.006).

In a cohort study of adults with COVID-19 admitted to intensive care units at 68 US medical centers, CKD was observed in 18.4% of patients at the time of admission to the intensive care unit [34]. In our case, in 58 patients (12.47%), CRF acted as a concomitant disease and demonstrated an inverse relationship with thrombosis. In the group with DVT, CRF was less common (p=0.011). And among patients with venous stasis, only one person (1.79%) suffered from CRF, out of 12.47% of patients with CRF among the entire sample. Thus, CRF was a protective factor against DVT, that is, patients with CRF were less likely (by 61%) to suffer from DVT compared to those who did not have CRF.

One study reported that despite ongoing prophylactic anticoagulant therapy, DVT was detected in 40% of hospitalized patients with COVID-19, and in severe patients with COVID-19, the incidence of DVT reached 65%. [35]. In other studies, the incidence of DVT in intensive care patients with COVID-19 was 27% [36]. A meta-analysis showed that the combined incidence of pulmonary embolism (PE) and deep vein thrombosis (DVT) in patients with COVID-19 was 16.5% and 14.8%, respectively [37], despite anticoagulant therapy.

In a study conducted in Russia during USAS of the veins of the lower extremities, DVT was found in 15 patients (20%), the effect of spontaneous echo contrast (SC) in 53 (70.7%) patients, which indicated blood stasis and a pronounced decrease in venous blood flow velocity in common femoral veins. In most patients, thrombi were found in the tibial segment in 13 patients (86.7%) and in 2 patients (13.3%) in the femoral segment [38].

In our study, DVT was detected in 60 (13.8%) resuscitation patients who underwent ultrasonography of the lower extremities. In most cases, DVT was detected at the level of the tibial segment. And the effect of spontaneous contrasting, indicating the presence of venostasis, was detected in 56 (12%) patients. The effect of spontaneous contrast enhancement of vein lumen in patients with coronavirus disease is associated with increased viscosity, i.e., blood clotting, an increase in the frequency of thrombotic events and complications. Assessing the presence of spontaneous echo contrast (SC) on ultrasound is a promising strategy and may be a useful alternative to laboratory measurements [39].

According to the results of the meta-analyses carried out by Jean-François Llitjos et al. and Li J et al., high D-dimer was found to be a major risk factor for DVT and that patients at high risk of DVT are more likely to be admitted to the intensive care unit [40, 41]. This could be due to a syndrome of systemic inflammatory response to activation of blood coagulation, defined as a high level of fibrinogen. In our study, high levels of fibrinogen and D-dimer were found in COVID-19 patients with respiratory failure as an indicator of hypercoagulability. These indicators were significantly higher in the group of patients with thrombosis, so it is necessary to take into account their significance when prescribing DUS even when there are no clinical symptoms of DVT. Elevated D-dimer and DUS values will be able to timely detect asymptomatic DVT and prevent thromboembolic complications in intensive care patients with COVID-19 in the future. In patients at low risk of DVT, the diagnosis can be safely ruled out if D-dimer levels are normal. On the other hand, if in patients at high risk of DVT, D-dimer analysis will be insufficient and ultrasound examinations are necessary.

In our study, all patients were in intensive care and we also found a D-dimer cut-off value, which indicates thrombosis with high sensitivity and specificity.

Because clinical signs do not allow diagnosis of DVT to be assessed, D-dimer levels can be used in combination with other parameters to provide timely ultrasound, making our study unique.

The study has many strengths. Firstly, the study was conducted prospectively and there were many opportunities to investigate risk factors for thrombosis at a glance. Second, this study is the largest study to date in the Republic of Kazakhstan, specifically designed for the timely diagnosis of DVT and assessment of the frequency of DVT in patients with COVID-19 undergoing venous ultrasound. The high frequency of DVT found in our patients with severe COVID-19 who were on prophylactic treatment and the correlation with respiratory parameters and some important laboratory data suggest that they can be used as a screening tool for patients who should undergo ultrasound. In these patients, ultrasound can be considered a useful and reliable tool for the early detection of DVT.

The present study had several limitations. DUS has been performed in a limited number of patients, largely due to the lack of available resources to scan all patients with elevated D-dimer levels and associated risk of death. Moreover, DUS was performed earlier in patients with DVT, and patients without DVT received a higher dose of heparin, so underestimation of DVT can be suspected in some cases. This suggests that clinical and laboratory suspicion before the study is always required. Also, the study was limited to screening for asymptomatic DVT, so the incidence of PE and the role of D-dimer in the diagnosis of PE remain unknown. The limited sample size may have limited the significance of our results. As for chronic renal failure, in our case, patients with CRF were less likely (by 61%) to suffer from DVT, compared with those who did not have CRF (p=0.028). If the study included a large number of patients, chronic renal failure may have shown another statistically significant predictor of DVT, which is a limitation in our study.

Conclusion

Our study confirmed that COVID-19 is associated with a high incidence of deep vein thrombosis (13.8%) in critically ill patients, in line with global statistics. Significant risk factors for DVT in patients in intensive care with COVID-19 are older age, high levels of D-dimer, and comorbidities such as obesity, diabetes mellitus. The identified factors must be taken into account for the formation of high-risk groups of patients with the development of thromboembolic events.

The threshold value of D-dimer calculated by us (>-2.33 μg/ml) is a predictor of thrombosis, which is an indication for the timely appointment of ultrasonic scanning of deep veins of the lower extremities. Timely correction of thrombolytic therapy based on coagulogram parameters and ultrasound results will help reduce the number of thromboembolic events in critically ill patients with COVID-19

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Comparison of blood gas analysis parameters, biochemical tests and hematological parameters in geriatric patients admitted to the emergency department

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Abstract

Aim: The primary aim is to compare blood gas parameters (sodium, potassium, glucose, hemoglobin, hematocrit) with biochemical test and hospital hemogram results and thus to investigate the compatibility of blood gas estimation with other laboratory tests in geriatrics. The secondary aim is to compare the effects of these parameters on patient mortality.

Material and methods: Patients over the age of 65 who applied to the emergency department were included in our retrospective study. Statistical Package for Social Sciences (SPSS Inc., version 20.0; Chicago, IL) was used for statistical analyzes applying to the emergency department. Statistical significance was accepted as $p<0.05$.

Results: 102 patients were included in our study and 51.97% were male. 9.8% of our patients died. The diagnostic test performance analyzes of BG (blood gas) hemoglobin, hemoglobin, BG hematocrit, hematocrit, BG glucose, glucose, BG sodium, sodium, BG potassium, potassium in predicting mortality revealed with the AUC(area under curve) value being calculated as unsuccessful for BG hemoglobin, hemoglobin, BG hematocrit and hematocrit (AUC value: 0.47, 0.45, 0.46, 0.50). AUC (area under curve) value being calculated as weak for BG glucose, glucose and BG sodium (AUC value: 0.64, 0.61, 0.63 respectively). AUC value being calculated as medium for sodium (AUC value: 0.71).

Conclusion: There is no superiority of blood gas parameters over hematological and biochemical parameters in predicting mortality in the geriatric patient group. However, blood gas parameters can be used in patient management as they correlate with other laboratory tests.

Key words: blood gas parameters, geriatrics, sodium, glucose, hemoglobin

Introduction

It makes it easier for us to have information about blood gases, acid-base balance disorders, hypoxia, metabolic disorders, fluid-electrolyte disorders, which we frequently prefer in bedside analyzes [1,2]. Routine electrolyte measurements are made with auto-analyzers in hospital centers. Although it is predicted to give results within 15 minutes, this measurement sometimes takes longer time in terms of hospital conditions and density [3,4].

Adequate equipment and income are required to get results in a shorter time. The usability of blood gas parameters instead of hospital laboratory parameters in the treatment and management phase is not yet clear.

Many studies have been conducted to compare hemoglobin, hematocrit, sodium, potassium and glucose parameters to determine the reliability of blood gas analysis parameters, and it has been discussed in many studies whether hematological parameters and biochemical parameters are compatible with blood gas parameters [5-7].

Although there are studies conducted in patients admitted to the emergency department [1], patients hospitalized in the intensive care unit [6], and pediatric patients [8], there is no study comparing blood gas parameters and hospital laboratory parameters in geriatric patients, to the best of our knowledge.

Aim: The primary aim is to compare blood gas parameters (sodium, potassium, glucose, hemoglobin, hematocrit) with biochemical test and hospital hemogram results and thus to investigate the compatibility of blood gas estimation with other laboratory tests in geriatrics. The secondary aim is to compare the effects of these parameters on patient mortality.

Material and methods

Ethics

The instant study was carried out with the permission of the University of Karamanoglu Mehmetbey, Karaman Education and Research Hospital Ethics Committee (Date: 24/05/2022, Decision No: 2022-KAEK-154/9).

Study design

Patients over the age of 65 who applied to the emergency department of Karaman Education and Research Hospital between 01.03.2022 and 01.09.2022 were included in our retrospective study.

Study population

Patients over 65 years of age, who applied to the emergency department between 01.03.2022 and 01.09.2022, with negative Covid-19 PCR (polymerase chain reaction) were included in the study. All patients under the age of 65, trauma patients over the age of 65, patients who developed cardiac arrest and for whom we have data obtained after cardiopulmonary resuscitation, and patients with missing laboratory data were excluded from the study.

Data collection

Heparinized syringes were used for blood gas samples taken from patients admitted to the emergency department (PICO50 Arterial Blood Sampler – Radiometer Medical ApS, Brønshøj, Denmark). The samples were studied with the blood gas analyzes used by the hospital (Radiometer ABL 700 Blood Gas Analyzer, Radiometer Medical ApS, Brønshøj, Denmark). These blood gas analyzers were calibrated 6 times a day. Hematology analyzer was used for hematological samples (Abbott Cell-Dyn 3700 Hematology Analyzer, IL, USA). For biochemical data, ion selective diluted method was used (ARCHITECT ci4100 Clinical Chemistry Analyzer, IL, USA, using a 2P32 ICT sample diluent kit).

The blood samples for the blood gas parameters, the hematological parameters, and the biochemical parameters were separate blood samples taken simultaneously.

Using hospital system data, demographic characteristics, disease diagnoses, vital signs, saturation, respiratory rate, fever, blood pressure, background information, sodium, potassium and glucose values obtained from blood gas and chemistry laboratory, and hemoglobin and hematocrit values obtained from blood gas and hematology laboratory 30-day mortality was recorded. Length of hospital stay, 30-day mortality rates, intensive care stays, and ward were also recorded. Length of hospital stay and ward and intensive care unit admission rates were recorded using the hospital data system. According to their survival status, the patients were divided into two groups (those who died and survivors) according to the National death notification system in Turkey. The examinations and data of patients who attended the emergency department were used.

Statistical analysis

The categorical data was done using the fisher exact test and chi-square test. Quantitative variables were presented as median and interquartile range (IQR, 25th-75th percentile) values, and the Mann-Whitney test was used in analyzing the paired groups. The normal analysis of continuous data was done using the Shapiro-Wilk test. Spearman correlation analysis was performed for each parameter. During this analysis, the area under the curve (AUC) values were calculated, and the sensitivity, specificity, accuracy, and 95% confidence interval (CI) data were analyzed. The AUC values of the parameters were calculated and tested mutually for significance with the DeLong quality test. Statistical Package for Social Sciences (SPSS Inc., version 20.0; Chicago, IL) was used for statistical analyzes applying to the emergency department. Statistical significance was accepted as $p<0.05$.

Results

Demographic data, comorbidities, symptoms and examination findings

102 patients were included in our study, 51.97% of them were male. 9.8% of our patients died and 80% of the patients who died were women. No statistically significant relationship was found between comorbid diseases and mortality. While applying with the most complaints of fatigue; No statistically significant relationship was found between symptoms and mortality. A statistically significant relationship was found between high fever and low saturation and mortality ($p=0.003$, $p=0.016$, respectively). The relationship of demographic data, comorbidities, and symptoms with mortality is shown in Table 1.

Relationship between laboratory parameters, clinical outcome with 30-day mortality

When the relationship between blood gas parameters and hemogram and biochemical values was evaluated, it was observed that WBC and sodium elevation had a statistically significant relationship with mortality ($p=0.012$, $p=0.028$, respectively). There was no statistically significant correlation between blood gas parameters (BG hemoglobin, BG hematocrit, BG glucose, BG sodium, BG potassium, BG lactate) with mortality ($p=0.727$, $p=0.710$, $p=0.151$, $p=0.190$, $p= 0.365$, $p=0.099$, respectively). While 33.3% of the patients were admitted to the service; 22.5% of them were admitted to the intensive care unit. 43.47% of the patients admitted to the intensive care unit died ($p<0.001$). Laboratory parameters are specified in Table 2.

Correlation between BG parameters, hemogram parameters and biochemical parameters

There was a statistically significant, positive and strong correlation between BG hemoglobin and hemoglobin (Spearman correlation test, $r=-0.95$, $p<0.001$); BG hematocrit and hematocrit (Spearman correlation test, $r=0.93$, $p<0.001$);

Table 1 Demographic data, comorbidities, symptoms, examination findings and 30-day mortality of patients

Dependent: MORTAL	**Survivor**	**Non-Survivor**	**Total**	**p**
Age Median (IQR)	76.0 (70.0 - 82.0)	82.0 (74.2 - 82.8)	76.5 (70.0 - 82.0)	0.255
Gender (n,%)				
Female	41 (44.56)	8 (80.0)	49 (48.03)	0.072
Male	51 (55.44)	2 (20.0)	53 (51.97)	
Comorbidities (n,%)	27 (30.0)	2 (20.0)	29 (29.0)	0.769
Hypertension	63 (70.0)	8 (80.0)	71 (71.0)	
Diabetes Mellitus	27 (30.0)	3 (30.0)	30 (30.0)	
COPD	28 (31.1)	2 (20.0)	30 (30.0)	
CAD	39 (43.3)	2 (20.0)	41 (41.0)	
CHF	9 (10.0)	0 (0.0)	9 (9.0)	
CVD	13 (14.4)	1 (10.0)	14 (14.0)	
CRF	5 (5.6)	1 (10.0)	6 (6.0)	
Malignancy	6 (6.7)	1 (10.0)	7 (7.0)	
DVT	4 (4.4)	0 (0.0)	4 (4.0)	
Use of anticoagulants	50 (55.6)	5 (50.0)	55 (55.0)	
Symptoms	59 (65.6)	3 (30.0)	62 (62.0)	0.064
Breathness	31 (34.4)	7 (70.0)	38 (38.0)	
Chest pain	5 (5.6)	0 (0.0)	5 (5.0)	
Hemoptysis	1 (1.1)	0 (0.0)	1 (1.0)	
Cough	36 (40.0)	6 (60.0)	42 (42.0)	
Sore throat	32 (35.6)	1 (10.0)	33 (33.0)	
Weakness	75 (83.3)	7 (70.0)	82 (82.0)	
Physical examination Median IQR				
Fever	36.5 (36.3-36.8)	37.0 (36.6-37.7)	36.5 (36.3-36.8)	0.003
Heart rate	88.5 (77.0-101.8)	98.5 (93.0-109.0)	89.0 (77.0-103.0)	0.045
Respiratory rate	19.0 (18.0-21.0)	22.0 (20.2-23.5)	19.0 (18.0-22.0)	0.010
Systolic blood pressure	134.0 (115.8-150.8)	122.5 (102.5-149.5)	134.0 (114.0-150.2)	0.364
Diastolic blood pressure	72.0 (66.0-80.8)	66.5 (60.0-77.5)	71.0 (65.0-80.2)	0.152
PH	7.4 (7.4-7.4)	7.4 (7.3-7.4)	7.4 (7.4-7.4)	0.333
Carboxyhemoglobin	0.9 (0.6-1.2)	0.9 (0.8-1.0)	0.9 (0.7-1.2)	0.945
Saturation (%)	93.0 (91.0-95.5)	90.0 (88.5-91.8)	93.0 (91.0-95.0)	0.016

(COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; CHF, Chronic heart failure; CVD, cerebrovascular disease; CRF, Chronic renal failure; DVT, deep vein thrombosis)

Table 2 Laboratory parameters, clinical outcome and 30-day mortality of patients

Laboratory Parameters Median (IQR)	**Survivor**	**Non-Survivor**	**Total**	**p**
BG Hemoglobin (g/dl)	13.0 (10.9-14.5)	12.6 (11.5-13.6)	12.9 (11.0-14.4)	0.727
BG Hematocrit (%)	38.0 (32.0-43.0)	37.0 (33.5-40.2)	38.0 (32.2-43.0)	0.710
BG glucose (mmol/l)	137.0 (105.8-189.2)	181.5 (137.5-214.2)	142.5 (107.2-194.5)	0.151
BG Sodium (mEq/l)	136.6 (133.2-139.6)	138.0 (135.4-144.1)	136.9 (133.4-139.9)	0.190
BG Potassium (mEq/l)	4.2 (3.9-4.6)	4.2 (3.7-4.4)	4.2 (3.9-4.6)	0.365
BG Lactate	1.7 (1.3-2.1)	2.1 (1.6-2.8)	1.8 (1.3-2.2)	0.099
WBC (103µ/L)	8.6 (6.9-12.7)	12.6 (12.2-15.0)	9.0 (7.1-12.9)	0.012
Lymphocyte (103µ/L)	1.5 (1.0-2.1)	1.1 (0.9-1.2)	1.5 (1.0-2.0)	0.200
Hemoglobin (g/dl)	12.8 (11.1-14.5)	12.3 (11.2-13.4)	12.6 (11.1-14.5)	0.612
Hematocrit (%)	37.8 (32.9-42.4)	36.7 (35.4-43.1)	37.7 (34.0-42.5)	0.982
Glucose (mmol/l)	136.0 (104.0-185.8)	194.5 (127.5- 228.2)	140.5 (104.2-199.0)	0.237
Sodium (mEq/l)	137.0 (134.9-139.3)	140.2 (138.5-141.7)	137.0 (135.0-140.0)	0.028
Potassium (mEq/l)	4.4 (4.1-4.7)	4.4 (4.0-4.5)	4.4 (4.0-4.7)	0.686
C-reactive protein	41.0 (7.4-110.5)	61.0 (11.4-141.2)	41.0 (7.6-110.8)	0.525
Clinical outcome				<0.001
Discharge	45 (48.9)	0 (0.0)	45 (44.1)	
Ward admission	34 (37.0)	0 (0.0)	34 (33.3)	
Intensive Care Unit	13 (14.1)	10 (100.0)	23 (22.5)	
LHOS/day Median (IQR)	2.0 (0.0 - 8.0)	9.0 (6.5 - 10.0)	4.0 (0.0 - 8.0)	0.004

(BG, blood gase; WBC, white blood cell; LHOS, length of hospital stay)

Table 3 ROC analysis for blood gas parameters, hemogram parameters and biochemical parameters for 30-day mortality

Parameters	Cut-off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
BG Hemoglobin(g/dl)	15.3	20%	89.13%	16.67%	91.11%	0.47
Hemoglobin (g/dl)	10.8	90%	22.83%	11.25%	95.45%	0.45
BG Hematocrit (%)	31	90%	18.48%	10.71%	94.44%	0.46
Hematocrit (%)	34.3	90%	27.17%	11.84%	96.15%	0.50
BG Glucose (mmol/l)	169	70%	67.39%	18.92%	95.38%	0.64
Glucose (mmol/l)	174	70%	68.48%	19.44%	95.45%	0.61
BG Sodium (mEq/l)	137.4	70%	59.78%	15.91%	94.83%	0.63
Sodium (mEq/l)	140	70%	75%	23.33%	95.83%	0.71
BG Potassium (mEq/l)	3.36	100%	3.26%	10.1%	100%	0.41
Potassium (mEq/l)	4.45	50%	58.24%	11.63%	91.38%	0.46

(BG, blood gase; PPV, positive predictive value; NPV, negative predictive value; AUC, area under curve)

BG glucose and glucose (Spearman correlation test, r=0.91, p<0.001); BG sodium and sodium (Spearman correlation test, r=0.85, p<0.001) and BG potassium and potassium (Spearman correlation test, r=0.80, p<0.001).

The diagnostic test performance analyzes of BG hemoglobin, hemoglobin, BG hematocrit, hematocrit, BG glucose, glucose, BG sodium, sodium, BG potassium, potassium in predicting mortality revealed with the AUC(area under curve) value being calculated as 0.47(16.67%-91.11%) for BG hemoglobin, with a cut-off value of 15.3; 0.45(11.25%-95.45%) for hemoglobin, with a cut-off value of 10.8; 0.46(10.71%-94.44%) for BG hematocrit, with a cut-off value of 31; 0.50(11.84%-96.15%) for hematocrit, with a cut-off value of 34.3; 0.64(18.92%-95.38%) for BG glucose , with a cut-off value of 169; 0.61(19.44%-95.45%) for glucose , with a cut-off value of 174; 0.63(15.91%-94.83%) for BG sodium, with a cut-off value of 137.4; 0.71(23.33%-95.83%) for sodium, with a cut-off value of 140; 0.41(10.1%-100%) for BG potassium, with a cut-off value of 3.36; 0.46(11.63%-91.38%) for potassium, with a cut-off value of 4.45 (Table 3).

Table 4 Comparison of sodium AUC values (De-Long test) (p=0.145)

	Cut-off point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC
BG Sodium (mEq/l)	137.4	70%	59.78%	15.91%	94.83%	0.63
Sodium (mEq/l)	140	70%	75%	23.33%	95.83%	0.71

(BG, blood gase; PPV, positive predictive value; NPV, negative predictive value; AUC, area under curve)

When BG sodium and sodium values with high AUC values were compared with the De-Long test, the BG sodium AUC value was 0.63; sodium AUC value was determined as 0.71(p=0.145) (Table 4).

Discussion

In our study, a significant correlation was found between glucose, hemoglobin, hematocrit, sodium and potassium values in blood gas parameters taken in geriatric patients and laboratory analyzes. Considering their relationship with mortality, AUC(area under curve) value being calculated as medium for sodium, weak for BG sodium, glucose and BG glucose.

Our secondary aim was to compare the effects of these values on the prognosis. However, sodium was the only value that had a statistically significant relationship with mortality (p=0.028).

In the literature, different results were obtained in studies on blood gas parameters. In a prospective study by Uysal et al., 1094 patients were examined; Core laboratory analyzes of hemoglobin, hematocrit, sodium, potassium and glucose values and blood gas analyzes were found to be correlated, similar to our study [1]. In another study, laboratory and blood gas analyzes of 11000 patients were examined over a 5-year period; A correlation was found between blood gas calcium, sodium and potassium values and laboratory values [2]. In another study comparing the laboratory autoanalyzer and blood gas analysis, there was no statistically significant correlation between potassium values; there was a statistically significant relationship between sodium values [4]. In our study, BG potassium and laboratory potassium values were correlated. We found that BG sodium and BG glucose values and laboratory sodium and glucose values were associated with mortality.

In the literature, rather than the relationship between blood gas and laboratory parameters and mortality, the correlation of blood gas and laboratory parameters with each other and whether they can guide treatment has been discussed [5,6]. Gavala et al., in their study, suggested that blood gas parameters should not be used instead of hospital laboratory tests in guiding treatment [5]. In a study conducted by Servent et al. in 51 intensive care patients, they stated that treatment can be planned based on blood gas measurements [6]. The correlation of hemoglobin, hematocrit, glucose, sodium and calcium values in blood gas analyzes and laboratory parameters in our study showed that blood gas parameters can be used instead of laboratory tests in geriatrics. In a study with two hundred and nineteen data, no statistical significance was found in the comparison of laboratory hemoglobin values with blood gas hemoglobin values; There was a statistically significant correlation in the comparison of blood gas analyzes of sodium and potassium and hospital laboratory values [7]. In a retrospective study that included 1927 pediatric patients, it was concluded that blood gas parameters such as hemoglobin, hematocrit, sodium, and potassium could not adequately correlate with hospital laboratory parameters, and these parameters should not be used in patient management [8]. In a retrospective study of more than thirty thousand patients, hemoglobin, hematocrit, sodium, potassium, glucose values were compared between blood gas analyzes and hospital laboratory analyzes, similar to our study. A strong correlation was found in glucose, hemoglobin and hematocrit values [9]. We think that the correlation of each blood gas parameter with hospital laboratory analyzes in our study will contribute to the literature.

Limitations

In our study, the examinations obtained at the first application were used. Since the patients who applied to the emergency department were included in the study and not every patient was hospitalized, control tests could not be taken during the hospitalization period of the patients and an evaluation was not made accordingly.

Conclusion

There is no superiority of blood gas parameters over hematological and biochemical parameters in predicting mortality in the geriatric patient group. However, blood gas parameters can be used in patient management as they correlate with other laboratory tests.

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Fear of COVID-19 and mother to infant bonding in postpartum women: Comment

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Introduction

Dear Editor, we would like to discuss an article entitled “Investigation of the relationship between fear of COVID-19 and mother to infant bonding in postpartum women” [1]. Şanlı and Akbag investigate the relationship between postpartum mothers' attachment to their children and their fear of COVID-19. According to Şanlı and Akbag [1], women in the postpartum period should seek help from healthcare professionals beginning in the pregnancy phase to deal with their fear of COVID-19 and create secure and healthy mother-to-infant bonding.

The fear of COVID-19 is a significant issue, and there are numerous factors at work. People who have a history of fear or anxiety are less likely to have faith in the local healthcare system. During a crisis, people may use public health solutions more or less frequently depending on how much trust they have in them. During a crisis, people may use public health remedies more or less frequently, depending on how much they trust their local public health administration. The case of COVID-19 vaccine hesitancy is a good example [2]. Furthermore, the COVID-19 situation rapidly changed, and the pattern of fear toward COVID-19 and the vaccine usually changes over time [3]. As a result, a longitudinal study on COVID-19 fear and mother-to-infant bonding in postpartum women may be beneficial in providing a clearer picture of the studied topic.

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