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RESEARCH ARTICLE

The Potential Therapeutic and/or Protective Effects of Steroid, PRP and Melatonin on Nise-Induced Hearing Loss In Rats

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noise, acoustic trauma, hearing loss, auditory brainstem responses, melatonin, platelet-rich plasma, steroid

Abstract

Introduction: To investigate the potential therapeutic and protective effects of steroid, platelet-rich plasma (PRP), and melatonin treatment on noise-induced hearing loss (NIHL).

Methods: A total of 42 rats were divided into five groups: Group 1 did not receive any drug, Group 2 received methylprednisolone via the intratympanic route at 24 hours after acoustic trauma (AT), Group 3 received PRP via the intratympanic route at 24 hours after AT, Group 4 received intraperitoneal melatonin at 24 hours before AT, and Group 5 received intraperitoneal melatonin at 24 hours after AT. Two of the 42 rats were sacrificed and used for blood source material to prepare PRP. Each group was exposed to noise at the 105 dB sound pressure level for 12 hours to induce AT. Auditory brainstem responses (ABRs) were determined before AT and on days 1 and 28 after AT, and then histomorphological assessment was performed to identify cellular changes.

Results: In the ABR test performed on day 28 after AT, a statistically significant improvement was found in the hearing thresholds of all the four treatment groups compared to the control group ($p < 0.05$). The improvement in the melatonin groups (Groups 4 and 5) was statistically significantly better than in the steroid and PRP groups ($p < 0.05$). Although the hearing thresholds of the steroid group were better than those of the PRP group, this difference was not statistically significant ($p > 0.05$). As a result of the histopathological examination performed on day 28 after AT, cell loss after AT was statistically significantly reduced in all the experimental groups compared to the control group ($p < 0.05$).

Conclusion: Based on ABR testing and histopathological findings in a rat model, we conclude that melatonin may be effective in reducing NIHL and noise-induced cochlear damage.

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Introduction

Noise is defined as unpleasant and unwanted sound, and the noise-induced hearing loss (NIHL) is considered a serious occupational and environmental health problem.[1] NIHL is one of the most prominent reasons for sensorineural hearing loss (SNHL) in adults, while it also causes labor and economic loss.[2-3] According to the World Health Organization (WHO) review in 2015, it is estimated that almost 1.1 billion young people worldwide could be at risk of NIHL.[4]

The traumatizing noise may be repetitive, continuous, or pure impulsive. Continuous exposure to intense noise causes temporary threshold shifts (TTSs) and/or permanent threshold shifts (PTSs). In addition, acoustic trauma (AT), injury or damage to the inner ear caused by exposure to excessively loud noise over a short time can result in irreversible auditory damage due to the loss of hair cells in the organ of Corti and impaired cochlear microcirculation.[5] TTSs are not accompanied by cell death but mostly lead to stereocilia dysfunction and resolve within 24-48 hours. However, if the noise and/or exposure to AT persists, PTSs with cell death may develop and sensorineural hearing loss becomes irremediable.[6] In cases where auditory hair cells cannot be replaced, preventing hair cell death or a therapeutic intervention in the first 24 hours after AT is critical to maintain hearing ability.[7] The main goal in this treatment is to reduce oxidative stress and inflammation on the cochlea and to reestablish the cochlear microcirculation. For this purpose, a variety of pharmacological agents, such as corticosteroids, H1 antagonists, melatonin, thymoquinone, pentoxifylline, vasodilator agents, and volume expanders are used.[7-10]

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted from the pineal gland and is particularly involved in the regulation of circadian rhythms. It is also a powerful antioxidant that stimulates antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase (GSH-Px), and glutathione reductase.[11-13] Melatonin neutralizes toxic reactants and inhibits the production of reactive oxygen species (ROS); thus, it can make cell membranes more resistant to an oxidative attack and protect DNA damage resulting from oxidizing agents.[14,15] Ischemia-reperfusion injury that develops after AT and free oxygen radicals (FORs) formed in response to this injury has an important place in cochlear sensorial epithelial damage, resulting in NIHL.[16-18] The use of melatonin, an important FOR scavenger, can also prevent and/or correct noise-related cochlear damage and related hearing loss.[8-19] Glucocorticoid has been reported to be another pharmacological agent with protective and therapeutic effects after noise exposure.[8-20] Platelet-rich plasma (PRP) is also used for the same purpose.[21] In this study, we aimed to investigate the potential therapeutic and/or protective effects of melatonin, PRP, and steroid treatment on NIHL in rats through histopathological and electrophysiological evaluations.

Material and Methods

This study was approved by the Animal Experiments Ethics Committee of Ankara Training and Research Hospital and conducted in accordance with the ethical regulations of the Declaration of Helsinki.

Animals

Forty-two healthy mature female Wistar albino rats weighing between 220 and 270 g were obtained from the Animal Experiments Laboratory of Ankara Training and Research Hospital Training and Research Hospital. Forty rats were housed in separate cages located in a temperature-controlled room (20-22 °C) under a 12-hour light/dark cycle. The ambient noise level was kept at <50 decibel (dB) at all times. All the rats were provided with free access to food and water. The remaining two rats were sacrificed and used for blood source material to prepare PRP.

Experimental protocol

An otoscopic examination was performed, and the ear wax in the external ear canal was removed under a surgical microscope (Carl Zeiss, Oberkochen, Germany). The ears with normal tympanic membranes were included in the evaluation. Hearing levels were assessed using auditory brainstem responses (ABRs) in both ears of all the animals on day 0 (baseline) under general anesthesia. The ears with a hearing loss of >20 dB and those with otitis media were excluded. The experimental design of the study is given in Table 1. Using random number tables, the 40 rats were randomized into the following five groups:

Group 1 (control; n = 8): no drug given.
Group 2 (steroid group; n = 8): Methylprednisolone was given via the intratympanic (ITM) route at 24 hours after AT.
Group 3 (PRP group; n = 8): PRP was given via the ITM route at 24 hours after AT.
Group 4 (melatonin group; n = 8): Intraperitoneal melatonin was given at 24 hours before AT.
Group 5 (melatonin group; n = 8): Intraperitoneal melatonin was given at 24 hours after AT. To induce AT, each group was exposed to noise at the 105 dB sound pressure level (SPL) for 12
To induce AT, each group was exposed to noise at the 105 dB sound pressure level (SPL) for 12 hours. At 24-four hours after AT, hearing levels were measured and compared to the baseline levels using ABRs in both ears of all the animals. On day 28, the ABR assessment was repeated for all the animals, and hearing results were recorded. Then, the animals were sacrificed under general anesthesia, and their cochlear tissues harvested for histopathological assessment.

Table 1. Experimental design of the study

Groups	Basal	Acoustic trauma	Day 1	Day 28	ITM	ITM PRP	M1	M2
Group 1	+	+	+	+				
Group 2	+	+	+	+	+			
Group 3	+	+	+	+		+		
Group 4	+	+	+	+			+	
Group 5	+	+	+	+				+

Abbreviation; ITM: Intratympanic; PRP: Platelet-rich Plasma; Group 1: Controls; Group 2: ITM Methylprednisolone; Group 3: ITM PRP; Group 4: Intraperitoneal Melatonin at 24 Hours Before Acoustic Trauma; Group 5: Intraperitoneal Melatonin at 24 Hours After Acoustic Trauma.

Two rats outside the study group were sacrificed and used for blood source material to prepare PRP. Blood was obtained by cardiac puncture (3 mL) and treated with 10% sodium citrate in a tube. Then, a two-step centrifugation process (1,000 rpm for 15 minutes and 3,000 rpm for 10 minutes) was followed. Finally, the PRP concentrate was dissolved in phosphate buffered saline (1:1). The PRP concentration was administered into both ears of the animals using a dental injector at 24 hours after AT. All the rats in PRP group received a single dose of PRP via the ITM route until the middle ear was filled (approximately 0.1 mL).
Methylprednisolone preparation and treatment

A 40 mg/mL concentration of methylprednisolone (250 mg/4mL, prednol-L, Mustafa Nevzat, Istanbul, Turkey) was administered into both ears of the animals using a dental injector at 24 hours after AT. All the rats in steroid group received a single dose of methylprednisolone via the ITM route until the middle ear was filled (approximately 0.1 mL).

Melatonin preparation and treatment

This treatment consisted of 5 mg/kg of melatonin [Melatonin Powder (CAS 73-31-4), Hangzhou Hyper Chemicals Limited, Hangzhou, China] and was intraperitoneally administered to Group 4 on the day before AT and Group 5 on the day after AT.

Auditory brainstem response measurement

The ABR test (AC 40 Interacoustics, Denmark) was performed under general anesthesia induced using 6 mg/kg intramuscular xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey) and 60 mg/kg intramuscular ketamine hydrochloride (Ketalar, Pfizer Co., Istanbul, Turkey). Needle electrodes were used to collect ipsilateral auditory evoked potentials. The reference and ground electrodes were placed onto the vertex in the midline and the active electrodes on the mastoid bilaterally. The electrode-skin impedance was kept below 5 kV. Beginning at a SPL of 90 dB, the SPL of the stimuli was decreased in 10 dB increments until the ABR wave III response was no longer observable. A total of 500 clicks were applied at a rate of 11.4/s through insert earphones (ER-3A; Etymotic Research, Inc., IL, USA). Five waves were identified in waveforms.

Noise exposure

A total of 40 rats were exposed to white noise at 105 dB SPL for 15 hours. The white noise was generated using an IAC AC40 model audiometer and applied via two speakers located at a distance of 50 cm from the cage on both sides. All the rats were awake while being exposed to the noise. Sound intensity was monitored with a sound-level meter (Tromer, P.R.C.) positioned near the external auditory canal.

Histopathological assessment

After the rats were sacrificed under general anesthesia, the temporal bones were dissected macroscopically and preserved in formalin fixative. Then, the tissues were placed in 10% formic acid for decalcification at room temperature for 24 hours. The formalin-fixed paraffin-embedded blocks of cochlear samples were cut using a Leica RM2255 rotary microtome, and serial sections of 5 mm thickness were obtained. Finally, the sections were dewaxed, rehydrated, and stained with hematoxylin-eosin (H&E). For the qualitative and quantitative analysis of the outer hair cells (OHCs) and spiral ganglion cells (SGCs), images were taken using a light microscope under 40x magnification (Leica Aperio CS2, Leica Biosystems Ltd, UK). For each animal, three mid-modiolar sections were selected, including the basal, middle, and apical turns of the cochlea. The outline of Rosenthal's canal profile was then traced, and SGCs were counted from the base to the apex of the cochlea within each profile. The density of SGCs was expressed in an area of 10,000 μm2.Cell counting in SGCs was performed using the Virapath-3.0.14 cell nucleus artificial intelligence analysis program (Figure 1) (Virasoft, ARI 1 Teknokent, Istanbul, Türkiye).

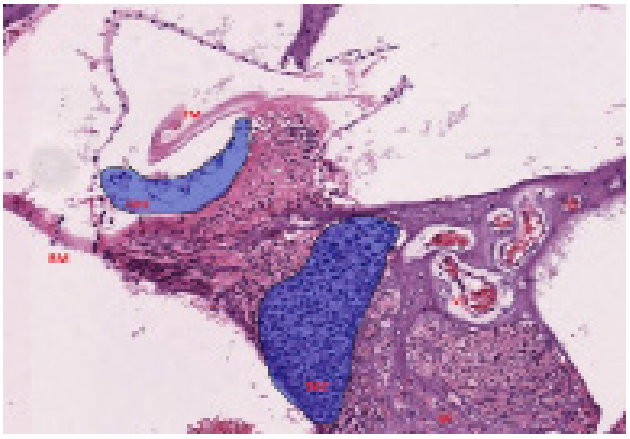

Fig. 1. Cell counting processes in spiral ganglion cells using the cell nucleus artificial intelligence analysis of Virapath software

Statistical analysis

SPSS statistical software program (SPSS, version 13.0 for windows; SPSS Inc, Chicago, Illinois, USA) was used to perform statistical calculations. Audiological results were compared with non-parametric two related (Wilcoxon) and two independent samples (Mann-Whitney U) tests. The density of SGCs (number of cell/10.000 μm2) calculated for each group was compared between the groups using one-way analysis of variance. Differences were accepted statistically significant at a p value of <0.05.

Results

Auditory brainstem responses

There was no significant difference between the groups in terms of the basal hearing thresholds before AT. Table 2 presents the me an ABR hearing thresholds for each group before AT and on days 1 and 28 after AT. No statistically significant difference was found in the three hearing threshold values between the right and left ears in each group. Therefore, the averages of the right and left ears were taken, and comparisons were made according to these values.

Statistically significant hearing loss was observed in all the groups based on the hearing thresholds obtained on day 1 after AT. According to the results of the hearing thresholds obtained on day 28 after trauma, there was a statistically significant improvement in the hearing thresholds of all the four treatment groups compared to the control group ($p < 0.05$).

The improvement in the melatonin groups (Groups 4 and 5) was statistically significantly better than in the steroid and PRP groups, while there was no significant difference between the two melatonin groups (Table 2). Although the hearing thresholds of the steroid group were better than those of the PRP group, the difference was not statistically significant ($p > 0.05$).

Histopathological findings at the end of week 4

As a result of the histopathological examination performed on day 28 day AT, the organ of Corti and spiral ganglion revealed a normal morphology in the steroid, PRP, and both melatonin groups. There was a significant decrease in the number of OHCs determined in the organ of Corti in the control group compared to the remaining groups (Figure 2, Figure 3).

The mean SGC density values determined separately for each group are given in Figure 4. Accordingly, the mean density of SGCs in all the four experimental groups was statistically significantly higher than in the control group ($p < 0.05$). There was no statistically significant difference between the remaining groups in terms of SGC density ($p > 0.05$).

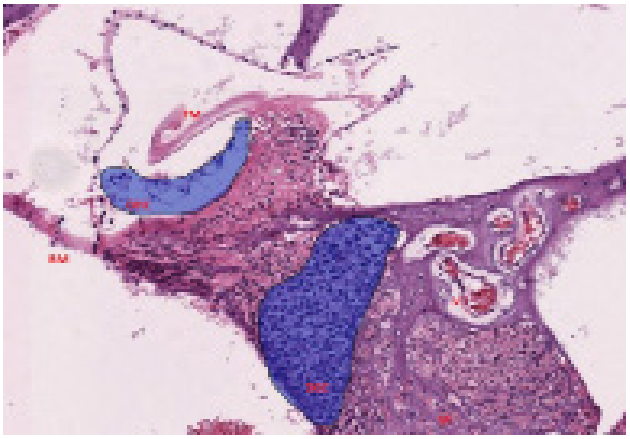

Fig. 2. Photomicrography of the organ of Corti and spiral ganglion in the control group. Note the decrease in the density of OHCs and SGCs. OHC: outer hair cell; SGC: spiral ganglion cell; BM: basilar membrane; TM: tectorial membrane

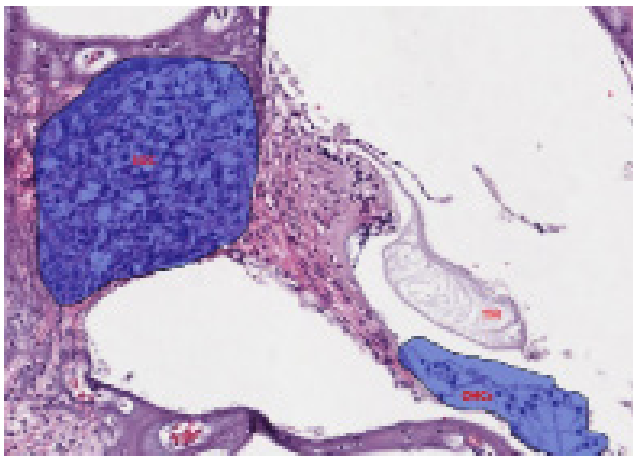

Fig. 3. Photomicrography of the organ of Corti and spiral ganglion in the group in which melatonin was administered before acoustic trauma. In this group, the organ of Corti and spiral ganglion have a normal morphology, and the density of OHCs and SGCs is higher compared to the controls. OHC: outer hair cell; SGC: spiral ganglion cell; BM: basilar membrane; TM: tectorial membrane; M: modiolus

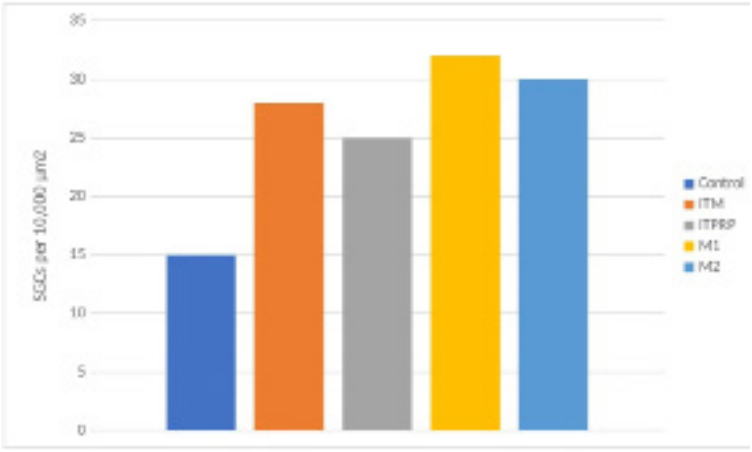

Fig. 4. Mean density of SGCs in each group

the remaining groups (Figure 2, Figure 3). The mean SGC density values determined separately for each group are given in Figure 4. Accordingly, the mean density of SGCs in all the four experimental groups was statistically significantly higher than in the control group ($p < 0.05$). There was no statistically significant difference between the remaining groups in terms of SGC density ($p > 0.05$).

Discussion

In this study, based on ABR testing and histopathological findings, we concluded that melatonin was effective in reducing NIHL and noise-induced cochlear damage in a rat model. Rats are often preferred in the investigation the effects of drugs administered for NIHL treatment.22 For the evaluation of hearing loss, both OAE and ABR methods are used.[7-9-20] In addition, hair cells are assessed under fluorescent or electron microscopy to determine which the effects of NIHL and drug treatment.[20] In the present study, we created an AT model in Wistar albino rats and used the ABR method to evaluate the effects of different drugs on hearing loss levels. We also performed histopathological assessment under light microscopy to evaluate the cellular effects on the cochlea. We used Virapath-3.0.14 cell nucleus artificial intelligence analysis program to determine the density of SGCs in the spiral ganglion in an attempt to reduce personal errors during cell counting.

NIHL can occur through two mechanisms including the direct mechanic trauma to the organ of Corti as a result of intense noise exposure and/

Table 2. ABR thresholds over time and between study groups

ABR threshold				p value - intragroup comparison (change over time)			
Groups	Basal (dB)	Day 1 (dB)	Day 28 (dB)	Total	Basal-Day 1	Basal-Day 28	Day1-Day-28
Group 1 (n=16)	6.5 ± 1.6	58.8 ± 8.3	30.0 ± 7.	<0.001	<0.001	<0.001	<0.001
Group 2 (n=16)	6.5 ± 1.3	57.5 ± 10.0	19.4 ± 6.8	<0.001	<0.001	0.003	<0.001
Group 3 (n=16)	6.9 ± 1.6	60.8 ± 10.3	21.8 ± 3.5	<0.001	<0.001	<0.001	<0.001
Group 4 (n=16)	6.6 ± 1.8	58.2 ± 7.9	11.7 ± 3.1	<0.001	<0.001	<0.001	<0.001
Group 5 (n=16)	6.5 ± 1.7	61.8 ± 11.3	11.3 ± 2.3	<0.001	<0.001	<0.001	<0.001
p value -intergroup comparison		NS	0.94	< 0.001 1 vs. 2 0.002 1 vs. 3 0.016 1 vs. 4 <0.001 1 vs. 5 <0.001 2 vs. 3 NS 2 vs. 4 0.046 2 vs. 5 0.031 3 vs. 4 0.006 3 vs. 5 0.004 4 vs. 5 NS			

Group 1: Controls; Group 2: Intratympanic Methylprednisolone; Group 3: Intratympanic Platelet-Rich Plasma; Group 4: Intraperitoneal Melatonin at 24 Hours Before Acoustic Trauma; Group 5: Intraperitoneal Melatonin at 24 Hours After Acoustic Trauma; NS: Not Significant

or metabolic stress due to the increase in oxidative metabolism in the inner ear.[6] Noise exposure reduces cochlear blood flow, causes hypoxia, ischemia, and inflammation, and promotes free radical production. Oxidative DNA then triggers apoptosis, resulting in the deterioration of stereocilia activity in the damaged cochlea, loss of hair cells, and SGHs, which leads to hearing loss.[11-18] Selective OHC loss most commonly occurs within 24 hours and continues for two weeks. Therefore, pharmacological agents used in the treatment of NIHL particularly aim at stopping and/or reversing the aforementioned pathogenetic process.5,8-10 In the current study, we investigated the efficacy of melatonin, PRP, and steroids with previously proven antioxidant and anti-inflammatory effects in preventing and reversing hearing loss in randomized controlled rat groups.

Steroids such as dexamethasone and methylprednisolone are the most popular agents used in the treatment of NIHL. For this purpose, not only intravenous but also ITM steroid administration is preferred.[5-8-10-20] Corticosteroids applied in the treatment of hearing loss due to AT act by binding to glucocorticoid receptors in the stria vascularis, organ of Corti, spiral ligament, and SGCs.[23] Corticosteroids increase cochlear blood flow and reduce hypoxia-ischemia damage by decreasing basal metabolic energy requirement and increasing anti-oxidant enzyme activity.[24] In a study in which dexamethasone was administered via mini-osmotic pumps connected to a cannula into the scala tympani in 26 male albino Hartley guinea pigs, Takemura et al. showed that dexamethasone provided significant hearing protection, and OHC loss was histologically less in the dexamethasone group relative to the control group.[25] In a study by Caliskan et al., the ITM and

systemic steroid administration was compared, and a statistically significant improvement was found in the group administered ITM dexamethasone at 5,000-6,000 Hz and in the group administered systemic methylprednisolone at 6,000-8,000 Hz compared to the control group.[26] In the present study, we applied a single dose of ITM steroid therapy. We achieved a statistically significant improvement in hearing thresholds determined by ABR following a single dose of intratympanic steroid administration compared to the control group. In addition, a single dose of ITM methylprednisolone injection administered following AT appears to reduce SGH loss. However, further studies are necessary to determine the most optimal dose, duration, and administration routes of steroid therapy. PRP has a positive effect on healing due to its high growth factor concentration.[27] In addition, previous animal studies have shown that these positive effects of PRP application also increase nerve damage regeneration.[27] In a study by Yurtsever et al., ITM PRP application was reported to be effective in cisplatin-induced ototoxicity and to reduce hearing loss and cochlear cell damage.[21] Similarly, in the present study, we found that a single dose of ITM PRP application after AT reduced the severity of hearing loss and cochlear cell damage. However, these positive effects of PRP were limited compared to melatonin and steroid treatment. Inner ear damage due to noise, drugs, radiation, and age-related hearing loss (ARHL) are all closely associated to the excessive production of free radicals that damage cochlear hair cells. Since the cochlea has a high aerobic metabolic rate, it is very sensitive to oxidative damage by ROS. The basal layer of the cochlea is the most affected part. Therefore, the main goals of treatment are to eliminate free oxygen radicals, which have an important place in the pathophysiology of hearing loss, and prevent oxidative damage. At this point, melatonin emerges as a very promising molecule. It has also been determined that melatonin has the same effect in the inner ear. Moreover, melatonin metabolites also function as ROS kidnappers so that more ROS can be eliminated, while lipid peroxidation is inhibited by melatonin.[11-14] A number of studies have suggested that melatonin can protect the inner ear from various types of damage. Animal studies on the efficacy of melatonin use in delaying ARHL and preventing cisplatin and gentamicin ototoxicity, radiotherapy-related hearing loss, and high-dose nicotine-induced cochlear hair cell damage, revealed its efficacy in hearing protection.[28-32] In a study by Serra et al., use of 10 mg/kg/day oral melatonin for a 12-month period in the murines was reported to be associated with significant increase in the viable cell density in the spiral ganglion and spiral ligament as well as in the distortion product otoacoustic emissions amplitude values. This suggests that less cell degeneration occurs in the cochlea of animals given oral melatonin, and ARHL development is delayed.[28] This is achieved by melatonin-mediated neutralization of oxidative stress during normal cell aging and the delay in apoptotic process. The glutathione enzyme system constitutes a protective mechanism against the development of NIHL. As the glutathione level increases in cochlear tissues after AT, oxidative stress and sensory epithelial damage decrease in the organ of Corti.33 Karlidag et al. revealed that melatonin applied during noise exposure minimized the decrease in the GSH-Px level in erythrocytes, reduced the production of malondialdehyde, a lipid peroxidation product, and contributed to the protection of hearing thresholds. In light of this information, the authors concluded that melatonin could play an effective role in protection from noise-related cochlear damage.[19] In another study, Bas et al. found that melatonin was more effective than dexamethasone or tacrolimus in reducing noise-related inner ear damage.8 Melatonin can be administered through the oral, IT, intramuscular, or intraperitoneal routes. Demir et al. determined that intratympanic melatonin administration of 0.1 mg/mL once a day for five days was effective against cisplatin-induced ototoxicity, and that transtympanic melatonin administration was effective in ototoxicity and in the treatment of sudden hearing loss.[34] This study has certain limitations. First, we determined hearing thresholds using ABR but did not evaluate frequency-specific hearing thresholds. The determination of frequency-specific hearing thresholds can be more beneficial in terms of revealing the efficacy of treatment. Second, we were not able to examine stereocilia because we performed the histological examination under light microscopy. Further histological studies using scanning

or transmission electron microscopy techniques are needed to provide a better understanding of the cell morphology. There is also a need to determine the optimum time, dose, and frequency of melatonin administration considering that results may differ when the dose and administration method is changed. Human trials to investigate the effect of melatonin against NIHL are also warranted.

Conclusion

We investigated the protective effects of melatonin in the treatment of NIHL by applying intraperitoneal melatonin before and after AT. Similar to previous studies, we found that this application was beneficial both electrophysiologically and histopathologically in the treatment of NIHL. Compared to both methylprednisolone and PRP treatments, the melatonin group had more positive results. However, we did not find a statistically significant difference between the administration of melatonin one day before and one day after AT. To our knowledge, current therapy for AT or noise-induced hearing loss is corticosteroid treatment. Accordingly, to be justified by human studies, our findings seem to indicate that melatonin may be considered an alternative to corticosteroid treatment against NIHL and AT.

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RESEARCH ARTICLE

The Role of 48-Hour CRP/Albumin Ratio in The Differential Diagnosis of Interstitial and Necrotizing Pancreatitis

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Abstract

Introduction: In this study, we aimed to examine the prognostic importance of the c – reactive protein (CRP)/albumin ratio in predicting acute necrotizing pancreatitis.

Methods: The study included 100 patients diagnosed with acute interstitial pancreatitis and 50 patients diagnosed with necrotizing pancreatitis between 2015 and 2020, all over the age of 18. **Results:** The CRP/albumin ratio was higher in the acute necrotizing pancreatitis group compared to the interstitial pancreatitis group (94.0 vs 34.0; p <0.001, respectively). Multivariable analysis revealed CRP/albumin ratio [OR: 1.075 (1.029-1.123), p = 0.001], source of infection [OR: 4.698 (2.078-10.620), p < 0.001], and lactate dehydrogenase [OR: 1.006 (1.002-1.010), p = 0.004] to be significantly predictive of developing necrotizing pancreatitis. A prediction value of CRP/albumin ratio >70.6 was found to be a significant marker in predicting necrotizing pancreatitis (Sensitivity: 76.0%; Specificity: 85.4%; AUC: 0.881; p < 0.001). In addition, to demonstrate the nonlinear relationship between CRP/albumin and the probability of necrotizing pancreatitis, cubic spline regression analysis was applied, showing that the probability of necrotizing pancreatitis increased in relation to the increase of the CRP/albumin ratio.

Conclusion: We conclude that the CRP/albumin ratio is a predictor of acute necrotizing pancreatitis. We believe that the CRP/albumin ratio is an inexpensive, sensitive, and easy-to-use predictor of acute necrotizing pancreatitis that can be used for both diagnosis and treatment follow-up.

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Introduction

Acute necrotizing pancreatitis is a severe form of acute pancreatitis associated with high morbidity and mortality that is characterized by the necrosis of the pancreatic parenchyma and/or the surrounding tissues.[1] It accounts for approximately 10% to 20% of all acute pancreatitis cases. About one-fourth of patients with necrotizing pancreatitis have a severe prognosis. The introduction of infection of the necrosis, gastrointestinal perforation, and bleeding into the clinical picture can increase the mortality rate up to 98%.[2] The prognosis in acute necrotizing pancreatitis cases is therefore fundamentally dependent on two factors: persistence of organ failure and secondary infection of pancreatic necrosis.

One of the important steps to reduce mortality in acute necrotizing pancreatitis is to understand the severity of the event and to plan the disease management accordingly before necrosis develops in and around the pancreatic parenchyma. This requires prognostically significant predictors of necrosis. Our review of the literature revealed that a large number of clinical and biochemical scoring systems have been assessed for this purpose. These primarily include the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, the Bedside Index of Severity in Acute Pancreatitis (BISAP), the Glasgow Pancreatitis Score, the Harmless Acute Pancreatitis Score (HAPS), the Japanese Severity Score (JSS), PANC3, the Pancreatitis Outcome Prediction (POP) score, the Ranson criteria, C-reactive protein (CRP), and systemic inflammatory response syndrome (SIRS).[3] However, among these scoring systems, only the Glasgow Pancreatitis Score and serum CRP levels provide pragmatic prognostic accuracy early on. Recently, the CRP/albumin ratio was used to predict the prognosis of acute edematous pancreatitis.[4]

Recent studies have used the CRP/albumin ratio as a prognostic marker for the severity of inflammation and mortality in chronic inflammatory diseases.[5-10] Since CRP is an acute-phase reactant that increases in inflammatory processes while albumin decreases in inflammatory processes, we believe that the CRP/albumin ratio can be used as a highly sensitive index for inflammatory diseases.

This study aims to investigate the prognostic significance of the CRP/albumin ratio in predicting acute necrotizing pancreatitis.

Materials and Methods

This retrospective study was conducted in the General Surgery Clinic of Ankara City Hospital between May 1 and June 1, 2020. The study was designed in accordance with the 2013 Brazil revision of the Helsinki Declaration and good clinical practice guidelines. The study was granted ethical approval by the Ankara City Hospital Ethics Committee.

The study included 100 patients diagnosed with acute interstitial pancreatitis and 50 patients diagnosed with necrotizing pancreatitis between 2015 and 2020, all over the age of 18. These cases were randomly included in the study in order of application.

The exclusion criteria were as follows: (a) being diagnosed with acute pancreatitis but refusing hospitalization, (b) being discharged during hospitalization of one's own accord, (c) having a preliminary acute pancreatitis diagnosis but dying before confirmation, (d) having non-pancreatitis-related infection foci, (e) severe malnutrition, (f) malignancy, (g) rheumatological or other diseases associated with chronic inflammatory processes, and (h) receiving immunosuppressive therapy for any reason.

Acute pancreatitis was diagnosed according to the National Pancreas Foundation criteria based on medical history, physical examinationfindings, amylase and lipase levels greater than 3 times the normal values, and/or computed tomography findings.[11] Pancreatitis diagnosis was confirmed using ultrasonography, computed tomography, or magnetic resonance imaging.

Clinical findings (respiratory rate, heart rate, Glasgow Coma Score, and body temperature), demographic findings (age, gender, comorbid conditions, antibiotic use, recurrent/chronic pancreatitis status, infection foci, length of hospital stay, disease severity, complications, and clinical outcome), laboratory findings [complete blood count, biochemistry, CRP, and erythrocyte sedimentation rate (ESR)], and radiological findings were retrospectively recorded from electronic and clinical files. Disease severity is classified as mild, moderate, or severe depending on the absence or presence of organ failure and local or systemic complications. Moderately severe acute pancreatitis is associated with transient organ failure that lasts <2 days, while severe acute pancreatitis is defined by organ failure that persists for ≥2 days.[12]

The CRP/albumin ratio was calculated by dividing the CRP level directly by the albumin level. The 48-hour laboratory parameters of the patients [white blood cells (WBCs), neutrophils, lymphocytes, monocytes, hemoglobin, platelets, CRP, ESR, blood glucose, urea, creatinine, sodium, potassium, calcium, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), lactate dehydrogenase (LDH), total bilirubin, direct bilirubin, amylase, lipase, and triglyceride] were recorded from patient files.

Statistical Analysis

Categorical variables were presented in frequency tables. Continuous variables were presented (mean, standard deviation (SD), or median, and interquartile ranges between 25% and 75%, as appropriate. Binary comparisons of numerical variables not conforming to a normal distribution were carried out using the Mann-Whitney U test. A p-value of less than 0.05 was accepted to indicate a statistical significance The receiver operating characteristic (ROC) curve was used to evaluate the performance of CRP albumin ratio. Additionally, to show the non-linear relationship between CRP alb ratio and necrotizing pancreatitis, we used cubic spline regression analysis and plotted the relationship between CRP albumin ratio and probability of necrotizing pancreatitis patients. Stata 15.0 Mac program was used for conducting the statistical analysis to evaluate the findings obtained from the study.

Results

Clinical and demographic characteristics of the study population are presented in detail in Table I. A total of 150 patients (100 with interstitial pancreatitis and 50 with necrotizing pancreatitis) were included in the study. The patients with interstitial pancreatitis (60.1 ± 18.0 years) were older than those with necrotizing pancreatitis (51.6 ± 17.2 years) (p = 0.007). The two groups were not significantly different in terms of gender distribution [interstitial pancreatitis, 56% female (n = 56); necrotizing pancreatitis, 44% female (n = 22)] (p = 0.17). The two groups were similar in terms of the presence of any comorbidity [interstitial pancreatitis, 48% (n = 48), necrotizing pancreatitis, 42% (n = 21)] (p = 0.49). As shown in Table I, necrotizing pancreatitis patients had significantly higher 48-hour respiratory rate (p = 0.001), 48-hour heart rate (p = 0.010), 48-hour Glasgow Coma Score (p = 0.008), 48-hour fever (p = 0.004), antibiotic use (p = 0.010), recurrent pancreatitis (p = 0.003), chronic pancreatitis (p = 0.004), duration of levels (p < 0.001), stay in intensive care unit (p < 0.001), and complication rates (p < 0.001) compared to interstitial pancreatitis patients. Four (8%) necrotizing pancreatitis and 2 (2%) interstitial pancreatitis patients died during hospitalization; this difference was not statistically significant. Other clinical and demographic characteristics of the study population are presented in Table I.

All laboratory findings are presented in Table II. CRP / albumin ratio was significantly higher in the necrotizing pancreatitis group compared to the interstitial pancreatitis group (94.0 vs 34.0; p <0.001, respectively). Several inflammatory mar-

Table 1. Clinical and demographic findings of the study population

Variable	Interstitial pancreatitis n(50)	Necrotizing pancreatitis n(100)	p
Age, (year)	60.1 (18.0)	51.6 (17.2)	0.007
Female, (%)	56 (56.0)	22 (44.0)	0.17
Radiological imaging, (%)			
No imaging	9 (9.0)	0 (0.0)	0.040
Computed Tomography	85 (85.0)	50 (100.0)	
Magnetic resonance	2 (2.0)		0 (0.0)
Ultrasonography	4 (4.0)	0 (0.0)	
Presence of comorbidity, (%)	48 (48.0)	21 (42.0)	0.49
48th – h breath count, (%)			
<21	92 (92.0)	36 (72.0)	0.001
>20	8 (8.0)	14 (28.0)	
Heart rate, (%)			
<91	95 (95.0)	41 (82.0)	0.010
>90	5 (5.0)	9 (18.0)	
48th – h GCS, (%)			
15	99 (99)	45 (90)	0.008
<15	1 (1)	5 (10)	
48th – h high fever, (%)			
Yes	89 (89.0)	35 (70.0)	0.004
No	11 (11.0)	15 (30.0)	
Antibiotic use, (%)	83 (84)	49 (98)	0.010
Infection source, (%)			
Not detected	24 (24)	2 (4)	<0.001
Pneumonia	3 (3)	2 (4)	
Urinary tract	5 (5)	0 (0)	
Gastroenteritis	2 (2)	1 (2)	
Biliary tract	63 (64)	5 (10)	
Infected pancreatic necrosis	0 (0)	7 (14)	
Peripancreatic abscess	2 (2)	5 (10)	
Pancreatic necrosis	0 (0)	8 (56)	
Recurrent pancreatitis, (%)	16 (16.0)	19 (38.0)	0.003
Chronic pancreatitis, (%)	5 (5.0)	10 (20.0)	0.004
Duration of hospitalization, (day)	8.9 (5.3)	28.2 (27.8)	<0.001
Intensive care requirement, (%)	12 (12.0)	18 (36.0)	<0.001
Severity, (%)			
Mild	39 (39.0)	0 (0.0)	<0.001
Middle	56 (56.0)	36 (72.0)	
Severe	5 (5.0)	14 (28.0)	
Complication, (%)			
Not detected	43 (90)	7 (28)	<0.001
Pseudocyst	4 (8)	2 (8)	
Wall of necrosis		0 (0)	8 (32)
Thrombosis	0 (0)	4 (16)	
Extra pancreatic complication		0 (0)	3 (12)
Mortality, (%)	2 (2.0)	4 (8.0)	0.077

Numerical variables were expressed as mean ± standard deviation or median (min-max).
Categorical variables were shown as numbers (%). * p <0.05 shows statistical significance.
Abbreviations: GCS: Glasgow Coma Scale

Table 2. Laboratory findings of the study population

Variable	Interstitial pancreatitis 100	Necrotizing pancreatitis 50	p
White Blood Cell Count, x10³/mL	10.7 (5.3)	15.2 (5.5)	<0.001
Hemoglobin, gr/dL	11.8 (1.7)	12.6 (2.1)	0.007
Neutrophil, x10³/mL	10.4 (4.3)	13.8 (4.7)	<0.001
Lymphocyte, x10³/mL	1.7 (3.3)	1.8 (1.3)	0.84
Monocyte, x10³/mL	0.7 (0.4)	1.0 (0.5)	<0.001
Platelet, x10³/mL	238.5 (72.3)	279.0 (99.6)	0.005
Blood glucose, mg/dL	144.6 (52.4)	177.2 (75.2)	0.002
Urea, mg/dL	23.4 (15.2)	30.3 (27.0)	0.048
48th – h Creatinine, mg/dL	0.8 (0.3)	1.0 (0.8)	0.10
ALT, U/L	216.3 (248.0)	98.4 (152.1)	0.002
AST, U/L	257.6 (302.2)	131.4 (215.4)	0.009
GGT, U/L	305.9 (311.5)	186.0 (246.0)	0.019
Amilase, U/L	1550.2 (1158.7)	1229.9 (1190.2)	0.12
LDH, U/L	235.4 (132.3)	480.2 (350.8)	<0.001
Totat biluribine, mg/dL	2.0 (2.1)	1.1 (0.7)	0.003
Direct biluribine, mg/dL	1.2 (1.7)	0.5 (0.4)	0.002
ALP, U/L	177.2 (154.5)	102.6 (45.8)	0.001
Albumin, g/dL	3.4 (0.4)	3.0 (0.6)	<0.001
CRP, mg/L	123.4 (89.9)	291.2 (114.7)	<0.001
CRP/albumin	34.0 (1.3 – 100.7)	94.0 (36.4 – 174.6)	<0.001
Eritrocyte Sedimentation Rate, mm/h	34.1 (23.9)	47.0 (32.1)	0.12
Lipase, U/L	685.9 (665.8)	420.3 (350.5)	0.15
Trygliceride, mg/dL	180.4 (257.6)	350.4 (386.0)	0.042
48th – h calcium mg/dL	8.5 (0.4)	8.3 (0.9)	0.69
Sodium, mEq/L	138.2 (3.6)	138.1 (4.7)	0.88
Potassium, mmol/L	4.0 (0.4)	4.1 (0.6)	0.27
Calcium, mg/dL	8.3 (0.5)	7.9 (0.8)	<0.001

Numerical variables were expressed as mean ± standard deviation or median (min-max).
Categorical variables were shown as numbers (%). * p <0.05 shows statistical significance.
Abbreviations: ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, GGT: Gamma Glutamyl Transferase, LDH: Lactate Dehydrogenase, ALP: Alkaline Phosphatase, CRP: C–Reactive Protein

kers were significantly higher in the necrotizing pancreatitis group compared to the interstitial pancreatitis group [WBCs ($p < 0.001$), neutrophils ($p < 0.001$), monocytes ($p < 0.001$), platelets ($p = 0.005$), LDH ($p < 0.001$), CRP ($p < 0.001$)], whereas ALT ($p = 0.002$), AST ($p = 0.009$), GGT ($p = 0.019$), and albumin ($p < 0.001$) were significantly lower. Multivariable analysis revealed CRP/albumin ratio [OR: 1.075 (1.029-1.123), p = 0.001], source of infection[OR: 4.698 (2.078-10.620), p < 0.001], and LDH [OR: 1.006 (1.002-1.010), p = 0.004] to be significantly predictive of developing necrotizing pancreatitis. As shown in Figure 1, A prediction value of CRP/albumin ratio >70.6 was found to be a significant marker in predicting necrotizing pancreatitis (Sensitivity: 76.0%; Specificity: 85.4%; AUC: 0.881; $p < 0.001$). In addition, to demonstrate the nonlinear relationship between the CRP/albumin ratio and the probability of necrotizing pancreatitis, cubic spline regression analysis was applied, showing that the probability of necrotizing pancreatitis increased in relation to the increasing CRP/albumin ratio (Table III) (Figure 2).

Table 3. Detection of risk factors associated with necrosis pancreatitis by multivariable regression analysis

Variables	Odds Ratio	Std. Err.	z	P>z	95% C.I. lower	95% C.I. upper
Age	.9598371	.0259399	-1.52	0.129	.9103188	1.012049
CRP/Albumin	1.074986	.0238381	3.26	0.001	1.029265	1.122738
Female	10.40612	16.14939	1.51	0.131	.4969342	217.9109
Presence of comorbidity	.5065307	.5006747	-0.69	0.491	.0729869	3.515333
Infection source	4.697751	1.955032	3.72	0.000	2.078026	10.62011
Recurrent pancreatitis	11.19728	18.32343	1.48	0.140	.453097	276.7159
Chronic pancreatitis	584.1837	1313.316	2.83	0.005	7.127623	47880
Lactat dehidrogenaza	1.006039	.0021074	2.87	0.004	1.001917	1.010178
Calcium	.4171726	.3710082	-0.98	0.326	.0729976	2.384093
48th – h breath count	9.116213	19.52286	1.03	0.302	.137063	606.3294
48th – h high fever	1.252869	1.710829	0.17	0.869	.0862114	18.20733

Abbreviations: CI: Confidence Intervals,

Discussion

In our study, the CRP/albumin ratio was higher in the acute necrotizing pancreatitis group compared to the interstitial pancreatitis group. Multivariable regression analysis revealed the CRP/albumin ratio to be an independent risk factor associated with necrotizing pancreatitis. Cubic spline regression analysis indicated that the probability of necrotizing pancreatitis increased in relation tothe increasing CRP/albumin ratio. To the best of our knowledge, this is the first study to investigate whether the CRP/albumin ratio plays a role in predicting acute necrotizing pancreatitis. The CRP/albumin ratio has been used as a prognostic and predictive marker in many diseases in the literature. Initial publications often examined CRP/albumin in the context of malignancy[10-13-17] and septic shock,[6,18] whereas later studies investigated the role of CRP/albumin in different disease groups.[19-22] CRP is very valuable in acute response in inflammatory conditions due to its short half-life. CRP levelsthe treatment of acute pancreatitis cases.[23] Wang et al. showed that low albumin and high CRP were associated with poor clinical outcome.[24] Since necrotizing pancreatitis is a poor outcome of acute pancreatitis, CRP and albumin may have prognostic significance in predicting necrotizing pancreatitis. Kaplan et al. examined the prognostic significance of the CRP/albumin ratio in 192 cases of acute pancreatitis.

They found the CRP/albumin ratio to be higher in acute pancreatitis patients who died compared to surviving patients. The CRP/albumin ratio was found to be an independent risk factor for mortality in acute pancreatitis. They also found a positive correlation between CRP/albumin ratios and Ranson scores (the prognostic significance of which has been demonstrated for acute pancreatitis), the Atlanta classification, hospitalization time, CRP, and ESR.4 In our study, the CRP/albumin ratio was higher in the group with necrotizing pancreatitis, which is a poor clinical outcome of acute pancreatitis, compared to the interstitial pancreatitis group. Hence, taking into account both our results and those of Kaplan et al.,[4] it can be inferred that for worse clinical outcome in acute pancreatitis, the CRP/albumin ratio and its sensitivity will be higher. This is supported by our cubic spline regression analysis finding that the probability of necrotizing pancreatitis increased in relation to the increasing CRP/albumin ratio. are often used at admission and in the follow-up of An important advantage of our study compared to the study by Kaplan et al.[4] is the high number of cases of necrotizing pancreatitis, a rare complication of acute pancreatitis, as well as the finding that the CRP/albumin ratio is useful in thedifferential diagnosis of interstitial pancreatitis and necrotizing pancreatitis. A further advantage 8-hour CRP and albumin levels, whereas Kaplan et al. used admission CRP and albumin levels to calculate CRP/albumin ratios.[2] Patients with acute pancreatitis commonly present with dehydration and intravascular volume reduction due to excessive volume depletion. This can translate into a dilutional increase in the molecules in the intravascular space, and this may affect the results of the study.

Yılmaz et al. evaluated the CRP/albumin ratio of patients with moderate to severe acute pancreatitis. They found that the CRP/albumin ratio was higher in the 60 patients with severe acute pancreatitis compared to the 204 patients with moderate acute pancreatitis.[25] However, the relationship between CRP/albumin ratio and poor prognostic factors has not been studied in detail.

Apart from these studies, we did not find any studies in the literature that examined the relationship between acute pancreatitis and the CRP/albumin ratio. One study evaluated the CRP/albumin ratio in patients with severe acute ulcerative colitis, a disease that progresses similarly to

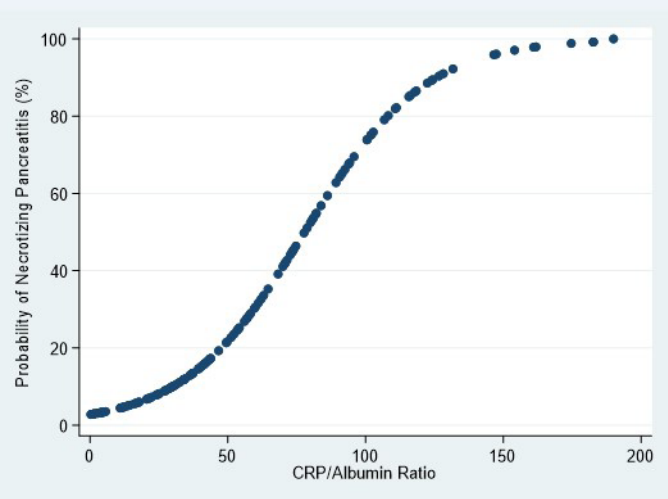

Figure 2. Cubic spline regression analysis for showing the probability of necrotizing pancreatitis increased in relation to the increasing CRP/albumin ratio

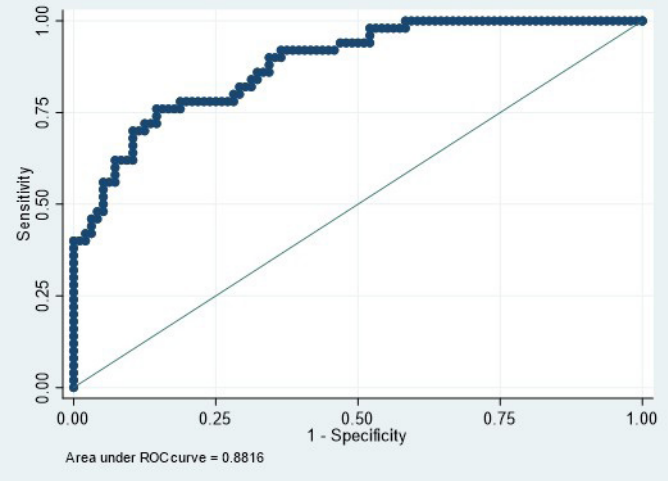

Figure 1. A prediction value of CRP/albumin ratio for predicting necrotizing pancreatitis

acute pancreatitis. The authors found that the CRP/albumin ratio can be used to differentiate severe ulcerative colitis from moderate and mild ulcerative colitis with a high value of the area under the curve.[26] A similar study investigated the role of the CRP/albumin ratio in Crohn's disease, a condition characterized by chronic inflammation, and found that the CRP/albumin ratio was associated with Crohn's disease activity.[19] The most important limitation of our study is its retrospective design. A prospective design may enable researchers to evaluate how the increased CRP/albumin ratio affects treatment response in necrotizing pancreatitis patients and the evaluation of its utility in treatment follow-up.A second limitation is that

albumin is a parameter closely associated with nutritional status. Although we excluded patients with any nutritional disorders indicated in their patient files from this study, there may have been some cases that were overlooked due to the retrospective design of the study. is that we calculated the CRP/albumin ratio using 4

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RESEARCH ARTICLE

Determinants Of Choosing Family Medicine As A Specialty For Young Doctors

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Abstract

Introduction: The reasons why family medicine specialists tend to choose this branch show differences in social and academic aspects. In this study, it was aimed to determine the reasons for choosing family medicine as the specialty of family medicine resident doctors and their concerns and fears while choosing their specialty. **Methods:** Our study is a cross-sectional observational study. 275 assistant doctors who started family medicine specialization training in Ankara between 2018-2020and are actively working constitute our study population. The participants were asked to score 0: the most ineffective, 10: the most effective, for the reasons for preferring family medicine consisting of 20 propositions and their concerns while choosing family medicine consisting of 7 propositions. **Result:** A total of 130 volunteer participants, who filled out the questionnaire completely, were included in the study. According to the survey results, the most important reasons to select family medicine specialty were; the desire to "spend more time with the family, the environment, and themselves", "less night and weekend shifts" and the desire for a "more easy and comfortable life". On the other hand, the main concerns of family medicine residents when choosing this specialty were "unnecessary rest reports demands" and "unnecessary medication demands" by patients. **Conclusion:** While it was determined that the personal factors of the residents were at the center of the reasons for choosing the family medicine branch, the characteristics and factors related to the discipline were the secondary preference reasons. On the other hand, it is necessary to take measures against alarming situations and develop new policies.

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Introduction

Specialization preferences of medical school graduates change periodically depending on numerous factors such as government policies, training opportunities, health system-related factors of the countries, and social prestige of the relevant discipline.1It is essential for every branch to know the expectations of future physicians' generation which are essential for their career choice. The reasons why family medicine specialists prefer this branch basically differ in terms of social and academic aspects.[2]

Along with the health transformation policies implemented in Turkey, family medicine practices have become widespread in the whole country under the name of "family physicians" in the last decade.3 Family medicine residency training started in Turkey in 1985, and to date, more than two thousand family medicine residents have received their specialty degrees from many family medicine departments and training clinics in Turkey. Family medicine residency training and working conditions differ in each country. The factors affecting the choice and causing concern should be determined specifically for the countries and, improvement and development should be made in the health and education system accordingly. For this reason, it is of great importance to bring the countries' situation on these issues and the solution proposals for possible problems to the international literature. Therefore, good practice examples can be provided.

There are numerous studies conducted to analyze the determinants of choosing family medicine for medical students in the United States, Germany, Canada, and Turkey.[1-2-4-7] Moreover, similar studies are presented targeting young doctors (residents and new graduates) in the United States and Australia.8–10There are limited studies targeting family medicine residents in Turkey.[11,12]

Determining the reasons why residents prefer family medicine residency training and their concerns about family medicine can give important results in terms of developing family medicine residency training programs and strengthening primary health care services.In this study, we aimed to determine the reasons of the residents just starting their family medicine residency training in Turkey for choosing a family medicine specialty and their concerns and fears in choosing the specialty.

Materials and Methods

Our study is observational and descriptive survey research which is conducted between 01/01-01/02/2020. Our study population consists of 275 residents starting their family medicine residency training between the years 2018-2020 in 10 educational institutions in the province of Ankara. It was aimed to reach the entire population within the scope of our study without considering any gender and racial-ethnic limitation and biases. 130 of 275 residents volunteered to participate in our study.

The parameters in the questionnaire were created by discussing in light of the information received from 6 academicians and approximately 40 residents in the family medicine clinic academic councils. After evaluating the comprehensibility and applicability of the questionnaire with a pilot study by the way of face-to-face interview method, the final version of the questionnaire was prepared with an online questionnaire preparati on program.The link created through the program was sent to 275 family medicine residents at the same time, first by phone and then by e-mail 1 month later.Informed consent was obtained from the participants at the beginning of the survey.

In the first part of the questionnaire, information about the location and duration of compulsory service after graduation (working as a physician before residency training), which may affect the sociodemographic characteristics and academic orientation of the participants, was questioned. In the second part of the questionnaire, the reasons for choosing family medicine were questioned, consisting of 20 propositions, and in the third part, their concerns about choosing family medicine were questioned, consisting of 7 propositions.The family medicine residents were asked to score between 0-10 points for the relevant propositions, with 0:the most ineffective, 10: the most effective. The scores given to each proposition were summed up separately for the total scores of the relevant propositions, and the preference rankings of the entire participant group were made. According to the first part which includes participants' factors of preferencefor Family Medicine branch, the first five most preferred parameters are gathered under two general headings. Accordingly, since the options "desire to spend more time with my family, myself and my surroundings", "less night

and weekend shifts", and "desire for a more relaxed and comfortable life" include situations such as "work-life balance", "family conditions", etc. we compiled and evaluated the relevant parameters under the title of "personal conditions". The options "to include a holistic approach to the patient" and "to include preventive health services" are special conditions for the Family Medicine Discipline, and we have gathered them under the title of "discipli ne-related factors". The categories of these factors of preferenceare classified and listed in Table 1.

Descriptive statistics of the data were analyzed together with the related questionnaire preparation program. The relevant parameters' median, minimum and maximum values or mean values were presented together with the standard deviation value by checking the conformity with the normal distribution of the continuous variables with the Skewness-Kurtosis test. Chi-Square tests were used to show relationships between variables. Descriptive statistics were done using frequency and percentage on categorical variables. A level of 0.05 wasconsidered significant. For the study, ethics committee approval (Document Date: 12/12/19, Document Number: E. Kurul-E 1-19-181) was obtained from the local research ethics committee and informed consent forms were obtained from the participants.

Results

A total of 130 volunteersfilled out the questionnaire completely, 92 females and 38 males, were included in the study. Participants were among the physicians who started to work as a resident in family medicine clinics throughout the province between 2018 and 2020. The return rate of the study was 47% of the entire population. The median age of the participants included in the study was 28, the lowest was 24 and the highest was 48. Of the participants, 47.7% (n:62) were single, 51.5% (n:61) were married, and 0.7% (n:1) were widowed. The median time of the participants' family medicine training was 4 months. In addition, 10% of the participants (n=13) stated that they worked as a resident in another specialty area before working as a family medicine branch was determined as 10 months.

The results of the 20 propositions that are among the reasons for the physicians to choose the family medicine branch and the findings of the 7 propositions that are among the concerns on choosing the family medicine branch are given in Table 2 and Table 3, respectively, starting from the one with the highest score.

Gender, age, marital status, and compulsory service experiences of participants are the independent variables. The median values of the propositions concerning factors and between the median values of all propositions of the reasons for preference are dependent variables. It was observed that the median values of the propositions of the reasons for preference according to gender and marital status did not differ.Based on Chi-Square tests, there are no significant relationships between the median values of all propositions of the reasons for preferenceandcompulsory serviceexperiences of participants ($p>0.05$). There are no significant relationships between the median values of all propositions concerning factors and compulsory service experiences of participants ($p>0.05$). Based on the Skewness-Kurtosis tests, the continuous variables showed normal distribution ($-3<$ skewness value<3; $-3<$kurtosis value<3). Based on Pearson Correlation tests, there are significant negative correlations between the age and themedian values of thefirst three propositionsof the reasons for preference, separately(Pearson correlation values= -0.504, -0.292, and -0.486, respectively; $p<0.05$). Based on Pearson Correlation tests, there are strong positive correlations between "the desire to devote more time to my family, environment and me" and "the desire for an easier and more comfortable life" (Pearson correlation=0.603, p=0.00); "inclusion of preventive health services" and "inclusion of a holistic approach to the patient" (Pearson correlation= 0.686, p=0.00)

Discussion

According to the results of this study, personal factors such as the desire "to spend more time with the family, the environment and themselves", and for this, "less night and weekend shifts", "more relaxed and comfortable life" and then discipline-related factors such as the desire to "provide holistic and preventive health care to their patients" are seen to be the reasons why the family medicine discipline is preferred.

In a study conducted with 237 senior medical school students in Turkey in 2017,the students' reasons for choosing a medical school, wishes on working with chosen institutions, specialties, and opinions on Medical Specialty Examination (TUS)

Table 1. Classification of Factors Making the Family Medicine Branch Preferable

Personal factors	The desire to devote more time to my family, environment, and myself Having less frequency of night and weekend shift The desire for an easier and more comfortable life Satisfactory income status Having support from my family Professional satisfaction Social prestige Being a comfortable section so I can get prepared for TUS again Having more opportunities abroad Recommendation of colleagues and the consulted family doctors Advice from Family Medicine professors at the School of Medicine
Discipline-related factors	Inclusion of a holistic approach to the patient Inclusion of preventive health services Having a high probability of working in the desired province as a specialist Providing an opportunity to work in the field, except for the hospital Having more opportunities to communicate with patients Having patients from all age groups Having more career opportunities Relatively shorter duration of residency training (3 Years) There are more quotas available in large cities such as Ankara

are questioned and it is seen that "the presence and number of duties" is the most effective factor after "their fields of interest " and "TUS scores" among the factors affecting students' choice of specialization. 7Moreover, in a study conducted with medical students from ten medical schools in 2019 in the USA, the lifestyle factors such as "having control of work schedule" and "having enough time offwork" were more important factors to choose a specialty for the fourth-year students than the first-year students, while specialty-related factors were more important for the first-year student than the fourth-year students.[13] It draws attention that in recent years physicians are choosing a specialty thatis more comfortable with fewer night and weekend shifts.

In a study conducted in 2013 to determine the factors affecting the residency preferences of students studying in different medical schools in the US and their interest in primary care medicine, it was found that the most important factor in choosing a specialty field was to enjoy the work done. When the reasons for preference of the students preferring Family Medicine residency in the first order are examined; it was determined that the factors such as "sparing time for family", "work/private-life balance" and "taking personal time outside of work" received high scores.[4]

In a survey conducted in 2011 with medical school students from five different medical schools in Germany, 7% of the students stated that they would prefer the family medicine branch, and the most influential factors of the preference reasons are "personal ambition" and "work-life balance". [2] In a survey study conducted with medical school students in Australia, the family medicine branch is the second preferred branch, and the most important factors in choosing it when compared to other branches are found in a related review to be "wanting to help people", "family circumstances", "flexibility in working hours", "residency duration time" and "family medicine education in medical school". [8-9] As a result of the survey conduc-

Table 2. Descriptive statistics: The Ranking of the Questions and the Median Values of the Scores According to the Total Points Given by the Family Medicine Assistants to the Factors Making Them Prefer the Family Medicine Branch

Factors Making the Family Medicine Branch Preferable	Total Points (n:130)	Median Value min:0; max:10	Maximum Value	Minimum Value	Mean± Std. Deviation
The desire to devote more time to my family, environment, and myself	1168	10	10	0	8.98±1.76
Having less frequency of night and weekend shift	1127	10	10	0	8.67±2
The desire for an easier and more comfortable life	1117	10	10	0	8.59±2
Inclusion of a holistic approach to the patient	849	7	10	0	6.53±2.8
Inclusion of preventive health services	823	7	10	0	6.33±2.9
Having a high probability of working in the desired province as a specialist	773	7	10	0	5.95±3.9
Providing an opportunity to work in the field, except for the hospital	744	6	10	0	5.72±3.4
Having more opportunities to communicate with patients	713	6	10	0	5.48±3
Satisfactory income status	686	5	10	0	5.28±2.9
Recommendation of colleagues and the consulted family doctors	669	5	10	0	5.15±3.2
Having patients from all age groups	626	5	10	0	4.82±3.3
Relatively shorter duration of residency training (3 Years)	583	4	10	0	4.48±3.6
There are more quotas available in large cities such as Ankara	566	4	10	0	4.35±3.5
Having support from my family	577	4	10	0	4.34±3.5
Professional satisfaction	551	4	10	0	4.24±3
Having more career opportunities	455	3	10	0	3.5±3.2
Social prestige	356	2	10	0	2.7±2.6
Being a comfortable section so I can get prepared for TUS again	345	1	10	0	2.65±3.4
Having more opportunities abroad	287	1	10	0	2.2±2.7
Advice from Family Medicine professors at the School of Medicine	173	0	10	0	1.3±2.1

Table 3. Descriptive statistics: The Ranking of the Questions and the Median Values of the Scores According to the Total Points Given by the Family Medicine Assistants to the Concerning Factors While Choosing the Family Medicine Branch

Factors to Worry on Choosing a Value Family Medicine Branch	Total Points (n:130)	Median Value min:0; max:10	Maximum Value	Minimum Value	Mean± Std. Deviation
Unnecessary health or rest report requests	1016	9	10	0	7.82±3
Unnecessary medicine requests	1015	9	10	0	7.81±3
The future of family medicine in our country	878	8	10	0	6.75±3.1
Family Medicine Residency is not being well-known	753	6	10	0	5.79±3.3
Having different models of residency training in full-time and contracted family medicine doctor	672	5	10	0	5.17±3.6
Being a fairly new branch	560	5	10	0	4.31±3
Lacking subspecialty opportunities	549	4	10	0	4.22±3.4

ted with medical school students in Canada, a positive relationship was found between "reasons for choosing family medicine" and "work-life balance".[6] These data show that "personal factors" are at the forefront in choosing family medicine specialty in different parts of the world. Although the study populations are different, it can be said that personal factors are more effective in choosing family medicine than academic factors, since the reasons for choosing family medicine of graduates from medical faculty are similar in the literature, and the reasons for the preference of resident doctors who prefer family medicine branch according to this study results.

Specialist doctors and preceptors who are positive role models in family medicine are important for attracting medical school students' attention to family medicine.[6-14-15] Having more roles in the education process as a Family Medicine branch in the early stages of medical school may encourage more students to choose family medicine specialization as a career. 1In a study conducted in 2019, the factors affecting the residency preference of 1814 newly graduated medical school students were examined with a survey study. As a result of this research, 39.2% of the participants stated that they wanted to choose the Family Medicine branch as their residency, and more than 90% of those who wanted to choose the family medicine branch stated that the most important factor in their preference was the support of the Family Medicine branch preceptors during their medical school years.[10] Again, in a study conducted with the students from the top ten medical schools in the US, it was seen that the most effective factor in choosing Family Medicine as a specialty was the presence of high-quality family medicine preceptors.[5] As a result of our survey, the factor of "the advice of Family Medicine preceptors in medical school" having the least effect on the choice of family medicine branch and the result of the study conducted in the US differ greatly. This may be caused by family medicine discipline in Turkey in the undergraduate curriculum not being addressed much or it lacking a standard format.

In another study conducted with medical school students in Germany, it was stated that adding Family Medicine as an elective course in medical school was effective in the preference of family medicine as a specialty, even among students who had no previous knowledge or interest.[16]

Again, the results of a survey study conducted in Germany indicate that the applied practice in Family Medicine program included in the discipline of Family Medicine in the early and late period of medical school will provide a great contribution.[17] In the literature, when medical school students were asked what affects their choice of specialty, it was seen that the existence of "role model family physicians with clinical experience" is one of the most important reasons for choosing family medicine, especially for students who want to choose primary care medicine.[18–20] In Turkey, the reasons for the low rates in this study may be the insufficient number of family medicine departments throughout Turkey or the preceptors in family medicine academic staff as role models, or the lack of adequate and effective communication between family medicine preceptors and medical school students within the scope of the undergraduate education program. Therefore, eliminating the shortcomings identified in light of these data may increase the preference rate for family medicine.

One of the issues that the resident doctors saw as worrying was the patients' inappropriate medication requests. The use of wrong, unnecessary, ineffective, and high-cost medications is a serious problem all over the world. The underlying causes of this may be caused by the physician, as well as by the patient. Increasing demands for unnecessary and inappropriate prescriptions by the patient is a problem faced by many doctors, especially family doctors. In separate studies conducted with physicians from three different branches; it was found that most frequently patients requested medicine prescriptions and laboratory tests, and least they requested consultation.[21-23] Many "rational choice" campaigns are launched to reduce unnecessary health practices around the world, to increase the awareness of physicians on this issue, to support physicians by organizing training, raise awareness of patients, and to ensure their active participation in the process; unnecessary examination and procedure lists were determined, and health literacy and media literacy issues for patients were brought up.[24] In a study conducted in Turkey, as in the rest of the world, it was found that patients' inappropriate demands were an effective factor in physicians' prescription of medicine and antibiotics without appropriate indications.[25] In a qualitative study conducted in İstanbul in 2017 to evaluate the problems of patients faced by primary care

physicians, the most common problems physicians encounter with patients are requesting unnecessary prescriptions, violence against physicians, problems related to examination order, after-hours examination requests by patients, and communication problems.[26] As a result of the study conducted for family medicine specialists and family medicine specialty students across Turkey in 2018 to evaluate patients' unnecessary prescription demands and outcomes in primary health care services, more than 95% of physicians occasionally or more frequently encountered the request of patients to prescribe off-label drugs, 4% of them rarely encountered, only less than 1% stated that they had never faced such a request. Again, more than 94% of physicians stated that they occasionally or often encounter patients' requests to issue an unnecessary rest report, and 5% of them rarely encounter; only less than 1% never encounter such a request.[27] When the results of these studies conducted in Turkey are examined, the most common problems faced by physicians are patients' requests for non-indication medicine prescriptions, unnecessary laboratory tests, and rest reports. In our study results, propositions such as "unnecessary rest reports" and "unnecessary medicine requests" are at the forefront of the concerns of family medicine residents when choosing this branch, similar to those in the literature. To eliminate such concerns, it would be beneficial to include education on health literacy and rational medicine use in informative activities for society. This situation is one of the most common problems faced by family doctors in Turkey as in the world and it may be considered alarming by the doctors within the scope of our study. Also, it was seen in the scores of the residents, who finished their compulsory service and they made up the majority of our participant group. The residents were also worried about this issue. The fact that they encountered this situation during their compulsory services and mostly in their first medical practices within the scope of primary health care services may cause this concern to be formed and settled.

In some countries, the fact that society does not recognize family medicine as a specialty is one of the most important of these problems. The physicians may think that in Turkey, the name "family physician" is given to the primary health care practitioner unit and the majority of physicians without a specialty degree are serving in this system, and the term "family medicine specialist" is not well-known by the society or it causes confusion in the meaning of the social prestige. Thus, sometimes it is difficult for family medicine specialists to express themselves as specialists to society. In addition, another problem is that society cannot understand the importance of family physicians' holistic and patient-centered approach. Therefore, it can be predicted that conducting studies to introduce the Family Medicine discipline as a residency branch for society will indirectly have a positi ve effect on the doctors who will prefer our discipline.

The number of physicians per capita is decreased in both Canada and the US, and this decline is expected to continue. When the growing population and the decreasing number of physicians are combined, it is expected that this decrease in the physician-to-population ratio will have negative effects on the health systems of both countries. Therefore, health policy planners will probably seek to expand the role of primary health care and especially family medicine. In order to support such an expansion, a proportional increase in the number of family doctors who have completed residency training domestically will be required6. Due to the low number of physicians per capita in Turkey, the number of family doctors is not at the desired level and each physician is required to deal with a larger number of requests such as unnecessary reports, medications, examinations, etc. and this may become a concern for physicians.

The results of our study on the concerns of family physicians when choosing this branch, which has not been studied much in the literature, have the potential to shed light on the dynamics of family physicians' relationships with their patients and suggest that developing policies to address the identified concerns and to solve these concerns will positively affect the preference rates and development of our discipline.

The low number of participants in our study prevents the generalization of our results. This situation has led to the fact that there are not enough group ratios to make comparisons and became one of the most important limitations of the study. 70.8 percent of the participants are women and all participants are a citizen of Turkey.Moreover, the possibility that physicians volunteering to participate in the study are the ones interested in the subject also raises the potential case of selection bias.

Our study includes national data that cannot be generalized. A sufficient number of people representing the universe could not be reached and the people reached could not be chosen randomly. Although it is known that the findings of our study may differ from country to country and each country should act according to its conditions, it is very important to know the family medical problems all over the world in terms of establishing exemplary practices against common problems, and this is both a limitation and a strong aspect of our study.

Conclusion:

According to the results of the study, it was determined that the reasons for preference depended more on personal factors and the characteristics and factors related to the discipline were the secondary preference reasons. "The advice of family medicine preceptors in medical school" has been the least effective suggestion in choosing family medicine. The introduction of family medicine discipline in medical professional education may increase the preference of this branch.On the other hand, "unnecessary rest reports" and "unnecessary medication requests" are at the forefront of family medicine residents' concerns when choosing this branch, and taking measures to address these concerns in the short and medium term and developing new policies in this regard may increase the preference of the family medicine discipline.

When the international and national literature is searched, there are studies on the factors affecting the medical school students' specialization choice in different countries and the factors that may be effective for them to prefer the Family Medicine branch. However, there are not so many studies on family medicine residents, and we hope that our study should help planning more comprehensive studies. Also, repetition of the studies at certain periods will contribute to the monitoring of the development of our discipline.

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RESEARCH ARTICLE

New Prognostic Markers in Acute Cholangitis Patients with COVID-19

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COVID-19; acute cholangitis; total bilirubin, prognostic markers

Abstract

Introduction: COVID-19 is the cause of a pandemic with high mortality rates that affect the entire world. In the present study, the importance of blood parameters was investigated in predicting the severity of the disease in patients diagnosed with both covid19 and acute cholangitis simultaneously. **Methods:** A total of 37 patient groups (n=37) who were diagnosed with both COVID-19 and acute cholangitis, a total of 38 patients in the control group (n=38) infected with Covid 19 and with no comorbidity, and 68 completely healthy control group (n) =68) were included in the study retrospectively and simultaneously. Those who had positive RT-PCR (Real-Time Polymerized Chain Reaction) test results were included in the study. The results of routine biochemistry, serology, hormone, and blood gas tests of the patients were compared with those of the control group. The Tokyo 2018 Criteria (TG18) were used in the diagnosis of acute cholangitis and disease severity grading. **Results:** The WBC(white blood cell), CRP(c-reactive protein), N/L Ratio(-neutrophile/lynfosite ratio), AST(Aspartat Aminotransferaz), ALT(Alanin Aminotransferaz), LDH(Laktate dehidrogenaz), GGT(Gama glutamil transferaz), ALP(alkalen fosfataz) total bilirubin, and direct bilirubin levels were higher in the patient group at statistically significant levels than in the control group ($p<0.001$). The albumin levels were lower than in the control group ($p<0.001$). Total bilirubin/lymphocyte, GGT/lymphocyte, ALP/lymphocyte ratios, and D-dimer parameters had the highest AUC (Area Under the Curve) values in ROC analysis (0.984, 0.924, 0.923, and 0.897, respectively). **Conclusion:** Total bilirubin/lymphocyte, GGT/lymphocyte, and ALP/lymphocyte ratios may be useful in predicting the severity of the disease in acute cholangitis cases developing with COVID-19 simultaneously.

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Introduction

The COVID-19 disease pandemic started to appear in China/Wuhan at the end of 2019 (COVID-19). Many people died since then [1]. With its high contagiousness, people had to be hospitalized and live in long-term quarantine. The virus still continues its disease-causing effects by undergoing various mutations. It was also given in many studies that COVID-19 and its variants cause high morbidity and mortality.[2-4] Studies are reporting the co-existence of COVID-19 and acute cholangitis.[5-6]

The symptoms of COVID-19 involve respiratory symptoms such as cough, fever, and shortness of breath. Many studies examined the effects of COVID-19 on other organs. Gastrointestinal symptoms such as diarrhea, vomiting, and hepatobiliary abnormalities were noted in patients with COVID-19.[7] The number of studies in which acute cholangitis accompanied by jaundice and COVID-19 coexistence is low.

In the present study, the purpose was to analyze and examine the effects of the development of acute cholangitis with the co-existence of COVID-19 on the clinical course of the disease, mortality, clinical classification scores, and biochemical parameters in the pandemic in which the virus, which is the causative agent of COVID-19, played a role in its etiology.

Materials and Methods

Study Design and Participants

The present study was designed retrospectively and approved by Ankara City Hospital/Turkey Ethics Committee.[2021-2047] The cases that were diagnosed with simultaneous COVID-19 and acute cholangitis in Ankara City Hospital between April 1, 2020, and September 01, 2021, were included in the study.

The demographic, clinical, laboratory data and radiological findings of the patients were obtained from the electronic medical records and hospital computer case record forms. The COVID-19 and concurrent cholangitis cases (n=37) were evaluated according to TG18(1) as mild, moderate, and severe. Also, the patients were divided into 3 groups as those who were infected with COVID-19 but without any comorbidity (n=38), the control group, and the completely healthy (n=68) control group. The baseline values of the patients at the time of admission were recorded by using the computer follow-up program, and intergroup comparisons were made. The albumin, total protein, glucose, urea, creatinine, ferritin, ALT, AST, ALP, GGT, LDH, total bilirubin, conjugated bilirubin, amylase, lipase, CK (creatinine kinase), CRP, procalcitonin, D-dimer, troponin, complete blood count, and N/L ratios were analyzed in all groups.

The demographic data of the patients, as well as clinical, laboratory, and radiological findings were evaluated to evaluate the acute cholangitis criteria. The severity of cholangitis, length of hospital stay, and mortality rates were examined in each category. The PCR+ test requirement was sought for COVID-19 contamination, and white blood test results were obtained for these patients. The TG18 was used for the diagnosis of cholangitis and grading of disease severity.

Diagnostic Criteria

Oro/nasopharyngeal swab samples for RT-PCR and routine blood tests were obtained from the patients for the diagnosis of COVID-19. Those with fever and respiratory symptoms according to the WHO (World Health Organization)Interim Guideline for the diagnosis of COVID-19, pneumonia findings on Thoracic CT, or clinical signs according to positive COVID-19 PCR results were also included.

The diagnosis of acute cholangitis was confirmed with the TG18 Criteria. For the diagnosis of the disease; *PART-A:* Systemic inflammation (fever; >38oCelsius, or WBC<4 or >10x1.000/ml and/or CRP>=1 mg/dL with laboratory data of inflammatory response). *PART-B:* Total bilirubin >=2 mg/dL. As abnormal liver enzyme values; ALP, GGT, AST, and ALT values of 1.5xSTD were accepted for the evaluation of cholestasis. PART-C: Biliary dilatation (abdominal CT, ERCP, MRCP, USG results) and biliary dilatation etiology (stenosis, stone, stent, carcinoma) were evaluated in the imaging evaluation.

Cardiovascular dyspnea, hypotension, dopamine 5 microgram/kg or any dose, noradrenali-

ne use), neurological dysfunction (blurring of consciousness), respiratory dysfunction (PaO2/FiO2 <300), renal dysfunction (creatinine>2.0mg/dL), hepatic dysfunction (PT-INR>1.5), hematological dysfunction (PLT<100.000/mm3) abnormal WBC (>12.000/mm3 or<4000mm3, high fever(>=39oCelsius), age (>=75) parameters were used for the grading of the severity of acute cholangitis disease.

Among the diagnostic criteria of the disease, in the criteria of systemic inflammation response (PART A), only other systemic inflammation responses without using fever>38oCelsius criteria, WBC, and/or CRP counts were used. Those who could be diagnosed with acute cholangitis without using fever criteria at the beginning were included in this study. Also, fever criteria were not used in the grading.

Statistical Analysis

The Statistical Package for Social Sciences for Windows, Version 22 (IBM, Armonk, NY, USA) was used in the statistical analyses of the study data. The Shapiro-Wilk test was used for the normality analysis of the variables. Descriptive statistical analysis was made by using the mean ± SD for normally distributed variables and median (interquartile range (IQR)) for non-normally distributed variables. The demographic and laboratory data were compared among the groups by using the Student's t-test for parametric variables and the Mann-Whitney U-test for non-parametric variables. The comparisons for categorical variables were made by using the Chi-Square test or Fisher's Exact test. Receiver Operation Characteristic (ROC) was constructed to analyze the effectiveness of disease severity. The optimal cut-off values of the D-dimer, Total bilirubin/Lymphocyte, GGT/ Lymphocyte, ALP/ Lymphocyte, WBC, and CRP were calculated by using the ROC Analysis. Statistical significance was taken as $p < 0.05$.

Results

The study included 37 hospitalized patients who were diagnosed with COVID-19 according to the WHO Criteria and also diagnosed with acute cholangitis, 38 patients who were infected with COVID-19 but without comorbidities, and a healthy control group of 68 participants. The demographic data of all cases that were included in the study, comorbidities, complaints of admission to the emergency department, and the degree of acute cholangitis according to Tokyo 2018 Criteria are given in Table 1.

Abdominal pain was the most common symptom in the acute cholangitis group at 3 different severities at admission to the hospital (p=0.686) along with jaundice (p=0.225). The data of the patients with comorbidities are given in Table 1. The most common comorbidities were cancer, systemic hypertension, and diabetes mellitus, respectively.

The hemogram and biochemical parameters and statistical analysis of the groups are given in Table 2. The WBC, CRP, N/L ratio, albumin, AST, ALT, LDH, GGT, ALP, total bilirubin, and direct bilirubin levels were higher in the patient group at statistically significant levels than in the control group (p<0.001). The albumin levels were found to be low (p<0.001) (Figure 1).

The ROC analysis of the routine blood parameters in predicting the prognosis in patients diagnosed with CoVID-19 and acute cholangitis:

The ROC Curve Analysis was used to determine the efficacy of various parameters in predicting the prognosis in the group diagnosed with COVID-19 and acute cholangitis (Figure 2). COVID-19 and acute cholangitis group was defined as positive, and the COVID-19 positive group without comorbidity wasdefined as the negative group. Total bilirubin/lymphocyte, GGT/lymphocyte, ALP/lymphocyte ratio, and D-dimer parameters in ROC analysis had the highest AUC (Area Under the Curve) values (0.984, 0.924, 0.923, and 0.897, respectively (p<0.001)). Albumin, on the other hand, had a low AUC value and was statistically not significant (p>0.05). AUC, optimal cut-off, sensitivity, and specificity values of the laboratory parameters are given in Table 3.

Table 1 Characteristics of the colangitis and control group

Variables	Total colangitis group (n=37)	Grade 1 (n=8)	Grade 2 (n=8)	Grade 3 (n=21)	p Value
Baseline characteristics					
Age, median (IQR),range, years	65 (50-78), 31-97	63 (53-78), 47-89	55 (38-72), 33-77	74 (52-84), 31-97	0.107
Gender, female/male	17/20	3/5	4/4	10/11	0.858
Outcome characteristics					
Mortality, (%)	16 (43.2%)	0	0	16 (76.2%)	-
Intubation	16 (43.2%)	0	0	16 (76.2%)	-
Comorbidities, (%)	31 (83.8%)	6 (75%)	8 (87.5%)	18 (85.7%)	0.359
Systemic hypertension, (%)	8 (21.6%)	1 (12.5%)	2 (25%)	5 (23.8%)	0.791
Diabetes mellitus, (%)	6 (16.2%)	1 (12.5%)	2 (25%)	3 (14.3%)	0.760
Cardiovascular or gan failure, (%)	3 (8.1%)	0	0	3 (14.3%)	-
Bile duct stones, (%)	4 (10.8%)	2 (25%)	1 (12.5%)	1 (4.8%)	0.306
Cancer, (%)	10 (27%)	2 (25%)	2 (25%)	6 (28.6%)	0.973
Symptoms at admission, (%)					
Fever	13 (35.1 %)	0	3 (37.5%)	10 (47.6%)	-
Jaundice	10 (27%)	1 (12.5%)	4 (50%)	5 (23.8%)	0.225
Dyspnea	5 (13.5%)	0	1 (12.5%)	4 (19%)	-
Vomiting/nausea	7 (18.9%)	2 (25%)	1 (12.5%)	4 (19%)	0.828
Tremor	5 (13.5%)	0	0	5 (23.8%)	-
Abdominal pain	17 (45.9%)	3 (37.5%)	3 (37.5%)	11 (52.4%)	0.686

Discussion

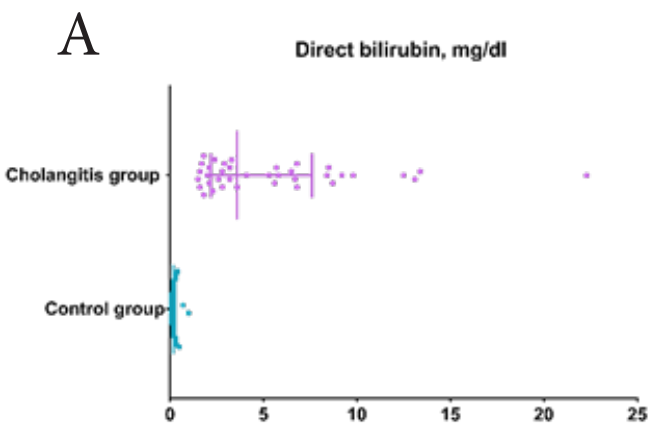
The COVID-19 pandemic continues to affect the entire world with different variations. Intra-abdominal organ involvement can be detected in COVID-19 cases, especially when it involves the lungs and multi-organ involvement. The present study, in which the data of COVID-19 patients who were PCR+ and also diagnosed with acute cholangitis were evaluated, was conducted in one single center. Few studies are reporting that acute cholangitis can develop in COVID-19 cases and affect the prognosis.[5-6] The present study is an example of detailed analysis in terms of providing a new alternative in the prognosis of cases that are diagnosed with COVID-19 and simultaneously with acute cholangitis.

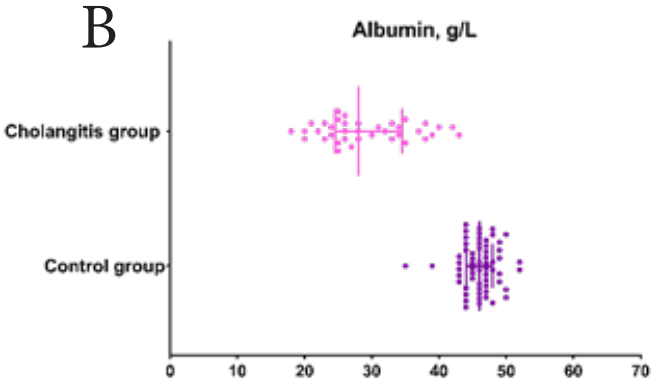
Table 2 Demographic characteristics of COVID-19 without comorbidity group, Cholangitis and control groups.

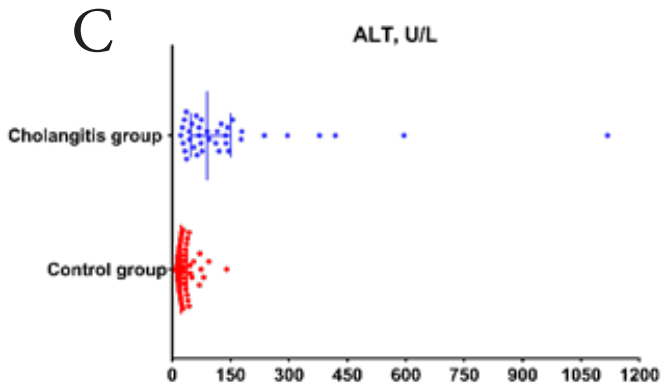
Parameters	Control group (n=68)	COVID-19 without comorbidity group (n=37)	Cholangitis group (n=37)	p Value
Age, years, range	40 (29-54), 18-79	44 (37-56), 25-73	69 (50-78), 31-97	<0.001
Gender, female/male	1/37	14/23	17/20	0.686
Amylase, U/L	59 (51.5-83)	65 (52-85)	40 (27-107)	0.101
Lipase, U/L	30 (25-37)	38 (31-45)	40 (21-111)	0.008
CRP, g/L	0.004 (0.002-0.007)	0.02 (0.006-0.08)	0.11 (0.022-0.156)	<0.001*
PCT, µg/L	0.03 (0.03-0.07)	0.03 (0.03-0.09)	0.44 (0.20-3.71)	<0.001*
WBC, 10^9/L	7.7 (6.8-9.2)	6.9 (4.5-8.8)	13.1 (10.2-16.9)	<0.001*
Neutrophil, 10^9/L	4.8 (3.1-7.7)	4.4 (2.8-7.0)	10.4 (6.3-15.6)	<0.001*
Lymphocyte, 10^9/L	2.1 (1.5-2.7)	1.3 (0.9-1.7)	0.9 (0.5-1.5)	<0.001*
N/L	2.1 (1.4-5.4)	2.9 (1.7-6.7)	8.3 (3.5-28.1)	<0.001*
PLT, 10^9/L	252 (203.5-308)	209 (162.5-256)	233 (113-355.5)	0.022
Hemoglobin, g/dL	13.8 (12.7-14.5)	14.5 (14-15.7)	10.5 (8.9-11.4)	<0.001*
Albumin, g/L,	46 (44-48)	44.5 (42-46)	28 (24.5-34.5)	<0.001*
Total protein, g/L	71 (68.5-73)	67 (65-70)	57.5 (46-62.5)	<0.001*
ALP, U/L	65 (60-76)	71.5 (58-83)	273 (183-520)	<0.001
AST, U/L	17 (14-23.5)	28 (19.5-38.3)	118 (72-267)	<0.001*
ALT, U/L	23 (16.5-37.5)	27 (21-47)	90 (48-150)	<0.001*
LDH, U/L	201 (174.5-244)	279 (216-338)	374 (244.5-722.5)	<0.001
GGT, U/L	20 (15-33.5)	25.5 (18.8-50.3)	256 (155.5-498.5)	<0.001*
D-dimer, mg/L	0.37 (0.22-0.49)	0.38 (0.27-0.67)	2.99 (1.3-7.3)	<0.001*
T.Bilirubin, mg/dL	0.6 (0.4-0.9)	0.5 (0.4-0.7)	4.8 (2.8-10.1)	<0.001*
D.Bilirubin, mg/dL	0.2 (0.1-0.3)	0.2 (0.1-0.2)	3.6 (2.2-7.6)	<0.001*
Urea, mg/dL	29 (22-39)	28 (24-38)	43 (24-75)	0.013*
Creatinin, mg/dL	0.74 (0.65-0.89)	0.85 (0.69-0.91)	0.77 (0.56-1.47)	0.402
Total bilirubin/LYM	0.4 (0.2-0.6)	0.4 (0.3-0.7)	7.4 (3.1-13.7)	<0.001*
GGT/LYM	10.5 (7.2-19.4	21.5 (11.3-63.7)	290.9 (139.2-692.7)	<0.001*
ALP/LYM	31.1 (23.1-46.9)	53.8 (34-87.8)	257.5 (164.8- 1063.6)	<0.001*

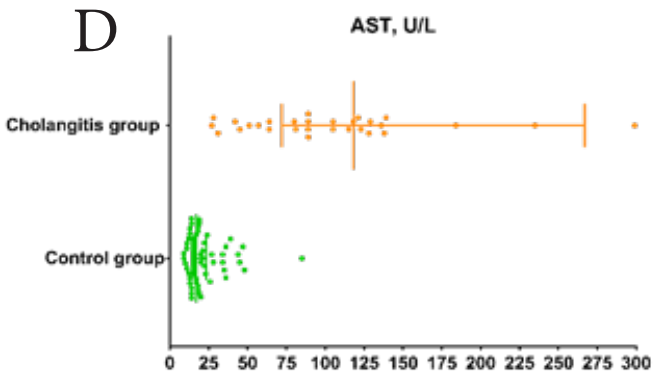
Data are expressed as median (IQR) for continuous variables. LYM: Lymphocytes. The comparison of COVID-19 without comorbidity and cholangitis groups highlighted with asterisk and significant according to Kruskal Wallis analysis.

*:p values less than .001 were considered significant highlighted in bold.


A
Direct bilirubin, mg/dl
Cholangitis group
Control group
0
5
10
15
20
25


B
Albumin, g/L
Cholangitis group
Control group
0
10
20
30
40
50
60
70


C
ALT, U/L
Cholangitis group
Control group
0
150
300
450
600
750
900
1050
1200


D
AST, U/L
Cholangitis group
Control group
0
25
50
75
100
125
150
175
200
225
250
275
300

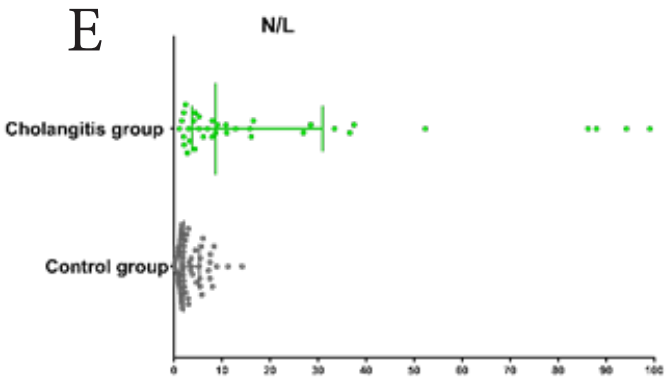

E
N/L
Cholangitis group
Control group

Table 3 The blood routine parameters in diagnosis of patients with COVID-19 with cholangitis on admission.

Variables	Cut-off value	AUC (95% CI)	Sensitivity (%)	Specificity (%)	p value
D-dimer	≥ 0.725	0.897 (0.803-0.992)	86.7	75.8	<0.001
Total bilirubin/LYM	≥ 1.748	0.984 (0.962-1.000)	93.3	90.9	<0.001 GGT/
LYM	≥ 73.6	0.924 (0.857-0.992)	86.7	87.9	<0.001
ALP/LYM	≥ 105.2	0.923 (0.854-0.992)	86.7	90.9	<0.001
WBC	≥ 10.05	0.727 (0.579-0.874)	73.3	84.8	0.002
CRP	≥ 0.062	0.726 (0.599-0.854)	66.7	72.7	0.002

AUC: Area Under the Curve; LYM: Lymphocytes. Asymptotic Significance Less Than 0.05 Were Considered Significant.

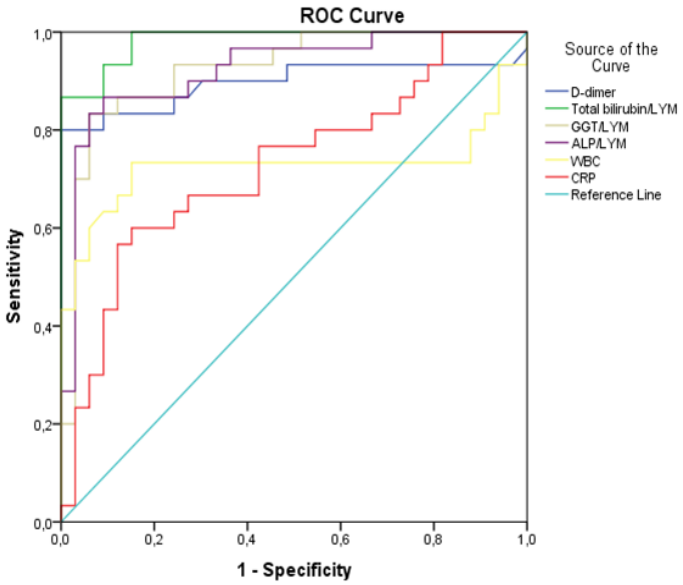


Fig. 2. The ROC curves of D-dimer, Total bilirubin/LYM, GGT/LYM, ALP/LYM, WBC and CRP in predicting COVID-19 with cholangitis on admission.

The release of TNF-alpha, IL-1, or IL-6, the pro-inflammatory cytokines common in patients with COVID-19 infection, especially in intensive care patients, causes hyperviscosity, hypercoagulopathy, thromboembolic event, which may deteriorate the condition of patients.[8] Proinflammatory cytokines also alter the function of hepato-biliary transporters along with the function of protective tissues in the bile ducts. The defect of the guard cells causes bile toxicity and may be a risk factor for chronic cholangitis.[9] The morbidity and mortality rate of cholangiopathy cases detected in COVID-19 cases can be reduced with early treatment.

Cholangiopathy is manifested by increased ALT, AST, GGT, LDH, T.Bil levels, and decreased albumin (ALB) levels in COVID-19 patients.[10-12] In the present study, AST, ALT, T.Bil, LDH levels were significantly higher in the COVID-19 + acute cholangitis group and the total protein and albumin levels were low. The findings were found to be compatible with the literature data. The abnormal detection of these biomarkers suggests that they can be used as the criteria for patients who may need intensive care in the treatment of COVID-19. Clinicians who treat COVID-19 should monitor the changes in liver biochemical indicators and detect patients with liver damage in the early period and initiate their transfer to Intensive Care Units. Increased D-dimer levels and lymphopenia were associated with higher mortality in COVID-19 patients.[13-14] In the study, D-dimer levels were found to be higher in the COVID-19 + cholangitis group. WBC, Neutrophil, lymphocyte, and NLR values were also significantly higher. WBC and NLR were shown to play roles in predicting the prognosis of chronic and acute inflammatory processes.[15] The presence of elevated WBC and NLR values in the coexistence of COVID-19 + and cholangiopathy may predict that the prognosis may be severe. In publications released on cholangiopathy, which may develop after COVID-19, it was associated with elevated liver enzymes such as ALT, AST, ALP, and GGT (16-17). These biomarkers have an important place in the diagnosis and follow-up. In a previous study, elevated liver enzymes, ALP, and GGT values were found in a case who developed liver failure (18). There was no case of hepatic failure in our cases.

Publications are reporting the optimum cut-off value of some serum biochemical parameters as the prognosis indicators in COVID-19 by using the severe disease ROC curve (19, 20). In the present study, in predicting the severity of the disease in COVID-19 + acute cholangitis patients in the ROC curve analysis, total bilirubin/lymphocyte, GGT/lymphocyte, ALP/lymphocyte rat ios, and D-dimer parameters had the highest AUC values (0.984, 0.924, 0.923 and 0.897, respectively ($p<0.001$)). Especially T.bil/lymphocyte, GGT/lymphocyte, and ALP/lymphocyte ratios can be useful in estimating the mortality and prognosis.

Conclusion

Elevated T.bil/lymphocyte, GGT/lymphocyte, and ALP/lymphocyte ratios may be useful in showing the severity of the disease in patients diagnosed with COVID-19 and acute cholangitis.

The authors declare that there is no conflict of interest between them.

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RESEARCH ARTICLE

Effect of Sodium-Glucose Cotransporter-2 Inhibitors on Circadian Blood Pressure Rhythm in Patients with Type 2 Diabetes Mellitus

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Abstract

Introduction: The aim of this study was to investigate the effect of sodium-glucose cotransporter-2 (SGLT2) inhibitors on circadian blood pressure rhythm in normotensive patients diagnosed with type 2 diabetes mellitus (DM) who were not on antihypertensives. **Methods:** The study included normotensive patients with type 2 DM who were initiated on SGLT2 inhibitors (empagliflozin, n=31; dapagliflozin, n=36) in addition to an antihyperglycemic agent. **Results:** Systolic blood pressure (SBP) and diastolic blood pressure (DBP) changed from a nondipper to a dipper pattern after treatment in 22.4% (n=15) and 25.4% (n=17) of the patients, respectively. Both SBP and DBP changed from a nondipper to a dipper pattern after treatment in 17.9% (n=12) of all patients. This change in circadian blood pressure was not significantly different for the dapagliflozin and empagliflozin groups ($p>0.05$). Fasting blood sugar and HbA1c levels significantly decreased in both groups after SGLT2 inhibitor treatment ($p<0.001$). Serum creatinine and spot urine microalbumin levels and the microalbumin/creatinine ratio decreased significantly in both groups ($p<0.05$). The posttreatment decrease in spot urine protein and creatinine levels was significantly higher in the dapagliflozin group compared to the empagliflozin group ($p<0.05$). **Conclusion:** The circadian blood pressure pattern changed from a dipper to a nondipper pattern in normotensive type 2 DM patients after they used SGLT2 inhibitors.

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Introduction

Hypertension and diabetes mellitus (DM) are associated with an increased risk of cardiovascular events and death.[1-2] Hypertension is a common comorbid condition in diabetes and affects 60% of DM patients.[3-4] This comorbidity further increases cardiovascular complications in the long run.[5] Uncontrolled nocturnal hypertension, in particular, has been associated with increased cardiovascular risk in patients with diabetes.[6] A nondipper pattern of blood pressure is a high-risk condition for arteriosclerosis, and therefore cerebrovascular events and cardiovascular disease,[7] and has been reported in patients with type 2 DM and insulin resistance. The management of nocturnal hypertension includes reducing circulating volume and preferably diuretics.[8] Sodium-glucose cotransporter-2 (SGLT2) inhibitors are mildly diuretic and produce specific effects on ambulatory blood pressure parameters. These inhibitors have been shown to improve glycemic control and lower blood pressure in type 2 DM patients.[9] The EMPA-REG OUTCOME trial showed that empagliflozin, an SGLT2 inhibitor, significantly reduced cardiovascular mortality by 38%.[10] The underlying mechanism by which SGLT2 inhibitors ameliorate cardiovascular disease is unclear; however, it may not be limited to metabolic parameters, body weight, and blood pressure. Although SGLT2 inhibitors have been reported to reduce systolic blood pressure by [2-5] mmHg, their effect on circadian blood pressure has not been fully elucidated.[11] Therefore, the aim of the present study was to investigate the effect of SGLT2 inhibitors on circadian blood pressure rhythm in normotensive patients with type 2 DM who were not on antihypertensives.

Materials and Methods

Study Design

This single-center prospective observational studywas conducted between 15 December 2019 and 15 June 2020 in the Ankara City Hospital Internal Medicine Clinic. The study was planned in accordance with the Declaration of Helsinki and was granted approval by the Ankara City Hospital Clinical Research Ethics Committee No. 1 (date 16/01/2020, decision number E1/182/2019).

Study participants

The study included normotensive (systolic blood pressure <140 mmHg and diastolic blood pressure <90 mmHg) type 2 DM patients of both sexes aged 18-80 years who used an antihyperglycemic and who were initiated on an SGLT2 inhibitor per indication. The exclusion criteria were as follows: history of using SGLT2 inhibitors, GFR <60 mL/min, genitourinary infection, hypertension and/or using antihypertensives (renin-angiotensin system blockers, calcium channel blockers, diuretics, alpha/beta blockers), malignancy, and immunosuppression.

Study Treatments

Patients who met the inclusion and exclusion criteria were randomized 1:1 to receive dapagliflozin 10 mg and empagliflozin 10 mg. Initially, two 24-hour ambulatory blood pressure measurements were planned: one before initiating SGLT2 inhibitors and one after 12 weeks of treatment. However, due to the coronavirus pandemic, there were delays in follow-up ambulatory blood pressure measurements (ABPMs). The average duration of follow-up was 19 weeks. Patients' diet and exercise habits remained unchanged.

Primary End Point

To demonstrate the change in circadian blood pressure compared to basal circadian blood pressure after 12 weeks.

Secondary End Points

To determine any changes in HbA1c, fasting blood glucose (FBG), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum urea, creatinine, spot urine protein, spot urine creatinine, spot urine microalbumin, and hemoglobin levels.

Ambulatory Blood Pressure Measurement

All patients underwent 24-hour ABPMs before being initiated on the study drug and after treatment for follow-up. They were asked to resume their normal activities. They were asked to be awake from 07:00 a.m. and 10:00 p.m. and to resume activities of daily living during this time and to rest and/or asleep between 10:00 p.m. and 07:00 a.m. ABPMs were performed using a GE Tenoport V (GE Tenoport V, Chicago, IL, USA). The device was programmed to perform a blood pressure measurementevery 30 minutes from 07:00 a.m. to 10:00 p.m. and every 60 minutes from 10:00 p.m. to 07:00 a.m.

Statistical Analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, IL, USA). The normality of data distribution was tested with the Kolmogorov–Smirnov test. Normally distributed numerical variables were presented as mean ± standard deviation, and nonnormally distributed numerical variables as median (minimum-maximum). Categorical variables were presented as numbers and percentages. Categorical variables were compared using chi-square and Fisher's exact tests. Student's t-test was used for the pairwise comparison of normally distributed numerical variables and the Mann–Whitney U-test for the pairwise comparison of nonnormally distributed numerical variables.The difference between before and after SGLT2 inhibitor treatment was analyzed by independent samples t-test and the Wilcoxon test for numerical variables and the McNemar test for categorical variables. Changes in laboratory and ABPMs between the treatment groups were compared by repeated measure mixed model analysis. $p<0.05$ (*) was accepted as statistically significant.

Results

The clinical and demographic findings are presented in detail in Table 1. The average duration of follow-up was similar between the empagliflozin and dapagliflozin groups, 19 weeks. The two groups were also similar in terms of the drugs used by the patients (Table 2). Blood pressure measurements before and after treatment according to the SGLT2 inhibitor groups are shown in detail in Table 3. Pretreatment APBMs were similar between the two groups. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) changed from a nondipper to a dipper pattern after treatment in 22.4% (n=15) and 25.4% (n=17) of the patients, respectively. Both SBP and DBP changed from a nondipper to a dipper pattern after treatment in 17.9% (n=12) of all patients. Among these, 66.6% (n=8) were on dapagliflozin and 34.4% (n=4) on empagliflozin. This change in circadian blood pressure was not significantly different between the two groups ($p>0.05$). In the dapagliflozin group, the mean daytime DBP significantly increased after treatment (72.6±8.5 mmHg vs. 75.2±7.9 mmHg, $p=0.034$). The remaining ambulatory blood pressure parameters did not significantly change. In the empagliflozin group, ABPMs did not significantly change after treatment ($p>0.05$). The pretreatment laboratory results were similar between the dapagliflozin and empagliflozin groups (Table 4). In the dapagliflozin group, mean FBG,creatinine, potassium, ALT, AST, GGT, ALP, and HbA1c levels significantly decreased, and mean urea and hemoglobin levels significantly increased after treatment ($p<0.05$, Table 4). In the empagliflozin group, mean FBG, ALT, AST, GGT, ALP, and HbA1c significantly decreased, and mean urea and hemoglobin levels significantly increased after treatment ($p<0.05$, Table 4). When the two groups were compared, only the change in creatinine levels was significantly different between the two groups, being higher in the dapagliflozin group (Table 4). The pretreatment spot urine findings of the two groups were similar. In the dapagliflozin group, mean spot urine protein, spot urine creatinine, and spot urine microalbumin levels and the spot urine microalbumin/creatinine ratio were significantly lower after treatment ($p<0.05$). In the empagliflozin group, mean spot urine microalbumin levels and the spot urine microalbumin/creatinine ratio were significantly lower after treatment ($p<0.05$). The posttreatment decrease in spot urine protein and creatinine levels was significantly higher in the dapagliflozin group compared to the empagliflozin group ($p<0.05$, Table 5)

Discussion

Our study is one of the few to demonstrate the effect of SGLT2 inhibitors on circadian blood pressure in normotensive type 2 DM patients.

In their meta-analysis, Zaccardi et al. showed that SGLT2 inhibitors decreased HbA1c by 7–10 mmol/mol (0.6–0.9%) independently of other antidiabetic treatments.[12] In our study, we observed a similar decrease in HbA1c levels. Our finding is consistent with the available literature.[13] However, SGLT2 inhibitors were not found to be associated with reduced blood pressure, HbA1c levels, or body weight.[14]

A dapagliflozin study by Lambers et al. reported a reduction in 24-hour SBP (mean 5.6±11.6 mmHg), daytime SBP (mean 8.8±12.25 mmHg), and nighttime SBP (mean 1.9±12.5 mmHg) [15]. In that study, the mean basal 24-hour SBP (131±12 mmHg), 24-hour DBP (77±7mmHg), daytime SBP (138±12 mmHg), and ni

Table 1. The distribution of demographic characteristics

Demographic characteristics	All subjects n = 67
Age (years)	52.4±8.4
Sex, n (%)	
Female	28 (41.8)
Male	39 (58.2)
BMI, kg/m2	29.0±3.5
Smoker, n (%)	
No	46 (68.7)
Yes	21 (31.3)
Alcohol use, n (%)	
No	63 (94.0)
Yes	4 (6.0)
Comorbidity, n (%)	
Hyperlipidemia	60 (89.6)
Thyroid disease	5 (7.5)
Anemia	3 (4.5)
Asthma	4 (6.0)
COPD	2 (3.0)
CAD	1 (1.5)
Other	
Crohn's disease	1 (1.5)
Alzheimer's	1 (1.5)
Epilepsy	1 (1.5)

Abbreviations: BMI: Body Mass Index, COPD Chronic Obstructive Pulmonary Disease, CAD coronary artery disease

Table 2. Distribution of drugs used before SGLT2 inhibitor treatment according to treatment groups

Drugs	Dapagliflozin n = 36	Empagliflozin n = 31	p
Metformin,n (%)	30 (83.3)	29 (93.5)	0.364
Statin, n (%)	14 (38.9)	8 (25.8)	0.304
DPP-4i, n (%)	7 (19.4)	7 (22.6)	0.772
Sulfonylurea,n (%)	7 (19.4)	6 (19.4)	0.999
Insulin, n (%)	8 (22.2)	5 (16.1)	0.758
Glitazones, n (%)	-	1 (3.2)	0.940
Glinides, n (%)	1 (2.8)	-	0.999

Categorical variables are presented as numbers (%).
Abbreviations: DPP-4i: Dipeptidyl Peptidase Inhibitor

pared to our basal blood pressure measurements. We ascribed this difference to the fact that Lambers et al. included patients on diuretics and with uncontrolled hypertension. The lack of nocturnal BP dipping in our patients may have been due to our having excluded patients on antihypertensives and those with uncontrolled hypertension.

An empagliflozin study by Tikkanen et al. reported a mean baseline 24-hour SBP of 131.3±13.0 mmHg and a mean baseline 24-hour DBP of 75.1±8.3 mmHg. They did not report data for other blood pressure parameters .[16] After treatment, they reported a reduction in 24-hour SBP (2.99±8.86 mmHg), 24-hour DBP (1.1±4.96 mmHg), daytime SBP (3.4±9.55 mmHg), daytime DBP (-1.28±5.41 mmHg), nighttime SBP (2.22±10.21 mmHg), and nighttime DBP (0.8±6.21 mmHg). It should be noted that their study included hypertensive and diabetic patients. Since the true effects of SGLT2 inhibitors on blood pressure are confounded by various variables in different populations, including BMI, ethnicity, basal blood pressure, and circadian blood pressure pattern, the results in the literature may need to be improved. Assessment of the effects of SGLT2 inhibitors on ABPMs requires ABPM data obtained after discontinuation of the drug. The improvement in blood pressure may result not only from the SGLT2 inhibitor but also from reduced sodium intake.

The literature reports conversion from a nondipper pattern to a dipper pattern in ABPMs after 14 days of dapagliflozin treatment.[17] In our study, change from a nondipper to a dipper pattern was seen in 17.9% of participants.

Posttreatment subgroup analyses by Baker et al. demonstrated that higher initial blood pressure (>140/90 mmHg) was associated with a greater reduction in BP .[18] In our study, we did not find any significant changes in 24-hour, daytime, or nighttime SBP or DBP values and ascribed this finding primarily to low baseline blood pressure and confounding factors such as diet and exercise.

The antihypertensive effect of SGLT2 inhibitors is usually attributed to natriuretic effects that are secondary to diuresis; however, the relevant mechanisms are not clearly understood. Multiple studies have sugges

Table 3. Changes in ambulatory blood pressure after treatment according to treatment groups

Spot Urine Findings	Dapagliflozin n = 36 *Before Treatment / After Treatment*		p	Empagliflozin n = 31 *Before Treatment / After Treatment*		p	Δp
24 hours							
SBP (mmHg)	119.3±15.9	122.2±15	0.141	120±12.4	120.9±14.6	0.739	0.515
DBP (mmHg)	71.2±8.1	73.3±7.5	0.088	74.0±8.5	73.5±7.6	0.729	0.164
Daytime							
SBP (mmHg)	121.1±16.9	124.7±15.3	0.095	122.4±13.3	122.8±14.3	0.872	0.369
DBP (mmHg)	72.6±8.5	75.2±7.9	0.034*	76.3±9.5	75.8±8.0	0.811	0.043*
Nighttime							
SBP (mmHg)	115.0±13.8	116.9±15.2	0.360	111.7±22.8	116.4±16.1	0.293	0.550
DBP (mmHg)	68.1±8.0	69.1±7.4	0.448	69.5±8.6	69.0±8.8	0.789	0.498
Systolic Dipping (%)	5 [(-5.5)-(15.9)]	5.5 [(-3.3)-(19.9)]	0.187	5.3 [(-12.2) -(21.6)]	5 [(-11.4)-(16)]	0.603	0.267
Diastolic Dipping (%)	6.7 [(-10.5)-(20.9)]	7.9 [(-5.3) -(20.3)]	0.239	6.8 [(-5.8)-(30.2)]	7.9 [(-8.4) -(27.5)]	0.814	0.574
Average heart rate (rpm)	76.9±8.5	78.4±7.6	0.792	77.4±10.0	79.0±9.6	0.257	0.914

Normally distributed numerical variables were presented as mean±standard deviation, and nonnormally distributed numerical variables as median (minimum-maximum).

Δp = significance of the difference between posttreatment changes in the two groups

Abbreviations: SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure

ted that these mechanisms are associated with diuresis, reduced weight, reduced vascular thickness, and reduced insulin resistance.[19]

The literature indicates that 52.8% of patients with DM have concomitant hyperlipidemia [20]. In our study, this rate was 89%. This may have been due to a lack of or inadequate antihyperlipidemic treatment due to reasons related to the physician or the patient. A study by Eriksson et al. on nonalcoholic fatty liver disease patients found that dapagliflozin significantly decreased ALT, AST, and GGT levels [21]. We similarly found a significant decrease in ALT, AST, GGT, and ALP levels after SGLT2 inhibitor therapy. This finding may be associated with hepatosteatosis and changes in weight. A study by List et al. demonstrated significant hematocrit elevation after 12 weeks of dapagliflozin treatment [22]. Merlin et al. found a 3-7% increase in plasma hemoglobin, albumin, and urea levels secondary to dehydration in patients on SGLT2 inhibitors.[23] In our study, mean hemoglobin, albumin, and urea levels significantly increased after treatment in both treatment groups. We evaluated this finding to reflect hemoconcentration secondary to diuresis. In our study, spot urine microalbumin and microalbumin/creatinine were reduced in both treatment groups. Reduced microalbuminuria may be due to decreased intraglomerular pressure after SGLT2 treatment. It has been reported that SGLT2 inhibitors reduce the risk of renal disease progression by 45%, regardless of atherosclerotic cardiovascular disease status.[24] The major limitations of our study are its single-center design (which limits the generalizability of the results), the small sample size, not having a placebo control group, and not having assessed dietary sodium intake. Moreover, we did not find a significant decrease in 24-hour, daytime, or nighttime SBP or DBP. Since follow-up ABPMs were made during the coronavirus pandemic, our results

Table 4. Changes in laboratory results after treatment according to treatment groups

Laboratory Findings	Dapagliflozin n = 36 *Before Treatment / After Treatment*		p	Empagliflozin n = 31 *Before Treatment / After Treatment*		p	Δp
Hemoglobin (g/dL)	14.6±1.5	15.1±1.3	0.002*	14.5±1.9	15.0±1.5	0.034*	0.786
HDL (mg/dL)	45.4±9.6	45.6±8.0	0.591	44.6±9.1	46.6±8.8	0.101	0.267
LDL (mg/dL)	126.7±29.7	112.6±28.9	0.085	115.6±22.9	109.7±33.0	0.301	0.428
Triglycerides (mg/dL)	167.5 (72 -656)	210 (68-425)	0.943	166 (55 -432)	164.5 (60 -835)	0.648	0.399
Fasting blood sugar (mg/dL)	175.5 (116-440)	125.0 (80-533)	<0.001*	161 (108-388)	119 (90-262)	<0.001*	0.755
Creatinine (mg/dL)	0.81±0.14	0.77±0.12	-0.009	0.81±0.13	0.81±0.15	0.779	0.049*
GFR(mL/min/1.73 m2)	95.2±9.5	95.0±19.1	0.981	97.8±9.6	97.1±11.1	0.525	0.800
Albumin (g/dL)	4.5±0.3	4.6±0.3	0.079	4.6±0.4	4.7±0.2	0.239	0.927
Urea(mg/dL)	31.9±7.5	35.1±9.0	-0.050	29.8±6.5	33.5±6.9	0.001*	0.706
Urea (mg/dL)	31.9±7.5	35.1±9.0	-0.050	29.8±6.5	33.5±6.9	0.001*	0.706
Sodium (mmol/L)	139.3±2.3	139.4±1.8	0.832	139.5±2.7	139.4±1.9	0.953	0.939
Potassium (mmol/L)	4.6±0.3	4.4±0.4	-0.050	4.6±0.4	4.4±0.3	0.036*	0.478
Phosphorus (mg/dL)	3.5±0.5	3.7±0.5	0.048*	3.5±0.5	3.8±0.8	0.040*	0.174
Magnesium (mg/dL)	1.8±0.2	1.9±0.2	0.008*	1.8±0.2	1.9±0.2	0.043*	0.516
Calcium (mg/dL)	9.5±0.4	9.8±0.5	0.048*	9.5±0.4	9.8±0.4	0.027*	0.880
ALT (U/L)	25 (9-164)	20 (13-100)	0.025*	3 (10-142)	27 (16-69)	0.022*	0.981
AST (U/L)	18 (10-117)	15 (4-63)	0.001*	22.5 (11-89)	18 (10-71)	0.002*	0.558
GGT (U/L)	27 (9-147)	24 (10-96)	0.011*	35 (16-159)	26 (16-107)	-0.031	0.591
ALP (U/L)	84 (55-159)	76 (53-163)	0.012*	90 (54-143)	82 (54-125)	0.035*	0.984
HbA1c (%)	9.2±1.6	7.7±1.5	<0.001*	9.1±2.0	7.6±1.2	<0.001*	0.690

Abbreviations: WBC: White Blood Cell, MPV: Mean Corpuscular Volume, RDW: Red Cell Distribution Width GFR: Glomerular Filtration Rate, HbA1c: Hemoglobin A1c, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, ALT: Alanine Aminotransferase, AST: Aspartame Aminotransferase, GGT: Gamma-Glutamyltransferase, ALP: Alkaline Phosphatase

Table 5. Changes in spot urine findings after treatment according to treatment groups

Spot Urine Findings	Dapagliflozin n = 36 *Before Treatment / After Treatment*		p	Empagliflozin n = 31 *Before Treatment/ After Treatment*		p	Δp
Spot urine protein (mg/L)	138.8 (14-390.3)	70.4 (9.8-340.0)	0.011*	83.9 (22.2-768.8)	62.8 (17.5-170.0)	0.722	0.043*
Spot urine creatinine (mg/dL)	132.5 (28.2-336.6)	81.1 (21.5-312.0)	0.001*	106.3 (21.6-179.2)	69.1 (24.8-182.5)	0.721	0.049*
Spot urinemicroalbumin (mg/L)	12.4 (0.1-76.1)	3.6 (0.6-34.3)	0.011*	7.2 (2.4-450)	3.8 (0.4-15.3)	-0.050	0.991
Urine protein/ creatinine (mg/g Cr)	104.7 (10.9-260)	86.8 (38-200)	0.778	78.9 (51-877)	90.8 (52.8-233)	0.139	0.737
Urine microalbumin/creatinine (mg/g Cr)	9.3 (0.3-75.8)	4.4 (1.4- 65.5)	-0.050	6.7 (3-513.4)	5.4 (1.5-32)	0.017*	0.352

Δp = Significance Of The Difference Between Posttreatment Changes In The Two Groups

p < 0.05 İndicates Statistical Significance

may have been affected by psychosocial risk factors such as stress, anxiety, and irregular sleep. The circadian blood pressure pattern changed from a dipper to a nondipper pattern in normotensive type ² DM patients after they used SGLT² inhibitors, but the mechanism by which this occurred is not clear. The changes in circadian blood pressure were not significantly different for the dapagliflozin and empagliflozin groups. Moreover, ²⁴-hour, daytime, and nighttime SBP and DBP did not significantly change. SGLT² inhibitors show more effective blood pressure reduction in poorly controlled hypertensive type ² DM patients.

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RESEARCH ARTICLE

Relationship Between Plasma Atherogenic Index And Coronary Slow Flow Phenomenon

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Atherogenic index of plasma, Biomarkers, Coronary slow flow, Frame count

Abstract

Introduction: Epidemiological studies indicated that patients suffering from coronary slow flow phenomenon (CSFP) are predisposed to dyslipidemia. However, there are limited studies evaluating the relationship between atherogenic index of plasma (AIP), which is a novel indicator of atherogenic dyslipidemia, and CSFP. This study aimed to investigate the prognostic role of the AIP in predicting CSFP among patients with undergoing coronary angiography. **Methods:** This retrospective study included 110 patients with CSFP diagnosed by methods of Thrombolysis in Myocardial Infarction (TIMI)-frame count (TFC) and 110 controls with normal coronary flow (NCF). AIP obtained as the base 10 logarithm of the ratio of triglycerides to HDL. **Results:** Mean AIP level was higher in the CSFP group than NCF group (0.6 ± 0.2 vs. 0.4 ± 0.2, $p < 0.001$). Multivariable regression analysis showed that AIP level (OR = 15.33, 95% CI = 4.11-57.18, $p < 0.001$), as well as neutrophil and platelets levels, were independent predictor of CSFP. The threshold value of the AIP in predicting CSFP was >0.7 with 64.5% sensitivity and 69.8% specificity (Area under the curve [AUC] = 0.714, $p < 0.001$). **Conclusion:** API was higher in CSFP patients and was determined as an independent predictor of CSFP. Prior to planned diagnostic coronary angiography, API exhibits significant diagnostic performance in predicting CSFP.

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Introduction

Coronary slow flow phenomenon (CSFP) is characterized by late opacification of contrast media into distal segment of one or more coronary arteries in patients with normal or near-normal coronary arteries during angiography.[1] There is still no clear consensus on the pathophysiology of CSFP. However, growing evidence suggested that some mechanisms, such as impaired lipid metabolism, atherosclerosis, endothelial or microvascular coronary dysfunction, platelet aggregation and inflammation, may play a role in the pathophysiology of CSFP.[2] Epidemiological studies indicated that patients suffering from CSFP are predisposed to dyslipidemia.[3-4] It is known that lipid metabolism is closely related to other mechanisms of CSFP such as atherosclerosis.[5] This is associated with the role of lipid metabolism in nitric oxide synthase activation, endothelial cell function, and induction of cytokines and coagulation factors.[6] Atherogenic dyslipidemia is characterized by elevated low-density lipoprotein cholesterol (LDL), apolipoprotein B and triglyceride levels and decreased high-density lipoprotein cholesterol (HDL) levels.[7] Atherogenic index of plasma (AIP) is a novel indicator of atherogenic dyslipidemia, and it is obtained as the base 10 logarithm of the ratio of triglycerides to HDL.[8] Meta-analysis studies indicated that AIP is an important predictor of cardiovascular diseases and events.[9-10] However, there are limited studies evaluating the relationship between AIP and CSFP.[11-12] These studies differ in the diagnostic performance of AIP in predicting CSFP. Therefore, more research is needed on the role of AIP on CSFP. Considering the relationship between lipid metabolism and other mechanisms of CSFP [13], we hypothesized that the AIP could be an important prognostic marker of CSFP. This study aimed to investigate the prognostic role of the AIP in predicting CSFP among patients with undergoing coronary angiography.

Material and Methods

This retrospective study included patients who had undergone diagnostic coronary angiograph in Ankara City Hospital Cardiology Clinic between January 2020 and January 2022. The study initiated with the approval of the Ankara City Hospital Ethics Committee (Date: 22.02.2023, Decision No: E1-23-3326) and was carried out in accordance with relevant ethical guidelines and the Declaration of Helsinki (revised in 2013, Brazil). The need for informed consent was waived by the local ethics committee due to the retrospective design. Based on a previous study, we determined the effect size of AIP as 0.74 in patients with and without CSFP (CSFP (+) = 0.70 ± 0.22 vs. CSFP (-) = 0.53 ± 0.24; p< 0.001).[11] Accordingly, it was determined by the G*Power program that the sample size should be at least 82 patients for each group with 5% alpha, 95% power, and 0.74% effect size.

Study population

A total of 4518 patients admitted to the hospital with stable or unstable angina pectoris and referred for diagnostic coronary angiography were evaluated retrospectively. The indication for diagnostic coronary angiography was positive ischemia in the exercise treadmill test or myocardial perfusion scintigraphy. Exclusion criteria were a history of heart failure, systemic inflammatory or autoimmune disease, thyroid dysfunction, coronary extasia, coronary artery stenosis (≥50%), valvular heart diseases, liver diseases, active hepatitis, malignancy, renal failure, lipid lowering drugs, and missing clinical data. After the exclusion process, a total of 225 CSFP patients with no stenosis in the main coronary arteries or their lateral branches greater than 2.0 mm were detected on coronary angiography results. For the control group, 250 subjects with normal coronary flow (NCF) findings on coronary angiography were selected. The groups were matched with the propensity match score using the 1:1 nearest neighbor matching method. The parameters used for matching were: age, gender, body mass index, comorbidities. Thus, 110 patients for each group were included in the analysis.

Analysis of patient data

The hospital's electronic information system and patient files were used to gather demographic and clinical data. In repeated measurements, blood pressure of >140/90 mmHg or use of antihypertensive drugs was defined as hypertension, and a fasting plasma glucose level of ≥126 mg/dL or use of antidiabetic drugs was defined as diabetes mellitus.

Blood samples were taken at the time of admission were measured using a Beckman Coulter LH 780 device (Mervue, Galway, Ireland). Levels of hemoglobin (photometrically), platelets (impedance method), C-reactive protein (immunoturbidimetric method), albumin (bromocresol green artery were evaluated. First side branch of right posterolateral artery for RCA, distal bifurcation for LAD, method), triglycerides and total cholesterol (enzymatic colorimetric method), and high-density lipoprotein (homogeneous enzymatic colorimetric method) were determined. The Friedewald formula was used to determine low-density lipoprotein levels. The AIP was calculated as follows: AIP = $\log_{10}$ (triglyceride / HDL ratio).

Coronary angiography

Angiographic data were analyzed in the cardiac catheterization laboratory by 2 cardiologists blinded to the clinical data of the patients. Patients underwent coronary angiograph through the femoral artery using the Judkins technique and were given an iopromide contrast medium (GE Healthcare, Cork, Ireland). Thrombolysis in myocardial infarction frame count (TFC) was used to evaluate coronary flow. In a nutshell, the cine frames number was recorded at 25 frames/s needed for the contrast to reach the standard distal coronary border point in the right coronary artery (RCA), left anterior descending (LAD) artery and left circumflex (LCX) artery were evaluated. First side branch of right posterolateral artery for RCA, distal bifurcation for LAD, and distal bifurcation of the major branch for LCX were defined as the distal ends of the coronary vessels. TFC is usually higher for LAD, which is longer compared to other main coronary arteries. Therefore, the TFC correction (cTFC) for LAD, obtained by dividing TFC by 1.7, was used.[14] The standard mean values of TFCs required for filling the coronary arteries have been previously defined.[14] In at least one coronary artery, CSFP was defined as ≥2 standard deviation of TFC values than published mean values.[14] The κ value for intra-observer and inter-observer variability between the two cardiologists was above 0.90 ($p < 0.001$).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows 20.0 (IBM Corp., Armonk, NY, USA). Based on the results of the Kolmogorov-Smirnov test, normally distributed numerical data were presented as mean ± standard deviation and non-normally distributed variables were presented as median values (25th-75th quartiles). For comparisons between groups, the Student t-test and Mann-Whitney U test were used according to the normality of the distribution. Categorical variables were expressed as numbers and percentages, and comparisons between groups were evaluated with Chi-square and Fisher exact tests. Multivariable logistic regression analysis was performed to identify any possible independent predictors of CSFP. Receiver operating characteristic (ROC) curve analysis was performed to evaluate diagnostic performance. Youden index method was used for threshold values. Values of $p < 0.05$ were considered statistically significant.

Results

The mean age of the 110 CSFP patients included in this study was 56.2 ± 8.3 years, the majority of them were male (70%). The baseline characteristics of the patients are reported in Table 1. The distributions of age, gender, and comorbidities were similar between the CSFP and NCF groups. Mean blood pressures and mean left ventricular ejection fraction did not differ significantly between the CSFP and NCF groups. Mean LAD-TFC (46.4 ± 2.5 vs. 22.4 ± 2.2, $p < 0.001$), mean LCX-TFC (34.5 ± 2.4 vs. 16.1 ± 2.1, $p < 0.001$) and mean RCA-TFC (31.3 ± 2.4 vs. 14.7 ± 2.2, $p < 0.001$) were higher in the CSFP group than NCF group (Table 1). The median neutrophil count (5.7 vs. 4.1 ×103 μL, $p < 0.001$), mean platelets count (256.5 ± 56.2 vs. 203.6 ± 42.4 ×103 μL, $p < 0.001$) and mean monocyte count (0.6 ± 0.2 vs. 0.5 ± 0.1×103 μL, $p < 0.001$) was higher in the CSFP group than NCF group, while mean lymphocyte count was lower (2.1 ± 0.6 vs. 2.3 ± 0.7 ×103 μL, $p = 0.024$). The levels of lipid profile also significantly differed between the groups ($p < 0.05$). Mean AIP level was higher in the CSFP group than NCF group (0.6 ± 0.2 vs. 0.4 ± 0.2, $p < 0.001$) (Table 2). Variables associated with CSFP (Tables 1 and 2) were considered as potential confounding factors. Among these factors, the components of the

AIP were not included in the regression analysis due to multicollinearity. Multivariable regression analysis showed that AIP level (OR = 15.33, 95% CI = 4.11-57.18, p < 0.001), as well as neutrophil and platelets levels, were independent predictor of CSFP. Accordingly, it was determined that a 1-unit increase in AIP level increased the probability of CSFP by 15.33-folds (Table 3). The diagnostic performance of AIP in predicting CSFP is shown in Figure 1. The threshold value of the AIP in predicting CSFP was >0.7 with 64.5% sensitivity and 69.8% specificity (Area under the curve = 0.714, p < 0.001) (Figure 1).

Discussion

The mechanism of CSFP, which has a wide presentation from mild chest pain to acute coronary syndrome, has not yet been elucidated.[15] The main mechanisms proposed for CSFP are thrombosis tendency, microvascular injury or disease, endothelial dysfunction and atherosclerosis.[2] Previous studies have shown that male gender, high body mass index, smoking, diabetes, hypertension and hyperlipidemia increase the risk of CSFP.[16-17] To more objectively assess the relationship between AIP and CSFP, we aimed to adjust for the effects of these potential confounding factors by creating a control group paired with propensity match score analysis. The main findings of the study were as follows: 1) AIP levels were higher in patients with CSFP. 2) Increased AIP was an independent predictor of CSFP. 3) The AIP score exhibited superior diagnostic performance in predicting CSFP.

In the present study, CSFP patients had lower HDL levels and higher triglyceride and LDL levels. This is consistent with the findings of epidemiological studies that patients with CSFP had a worsened lipid profile.[17] It has been shown that there is a significant correlation between hypertriglyceridemia and impaired coronary vasodilation in the absence of significant coronary stenosis.[18] Triglyceride-rich lipoproteins affect HDL levels and particle sizes, resulting in rapid catabolization of rich triglyceride and poor HDL-cholesterol ester particles. This atherogenic property contributes to atherosclerosis.[19] Dyslipidemia may cause in decreased aortic elastic properties. This result in impaired coronary blood flow. Increased reactive oxygen species and oxidized LDL induce vascular dysfunction and endothelial cell apoptosis.20 Small HDL and small dense LDL particles, which are more atherogenic than plasma LDL cholesterol, have limited use in clinical practice because of their cost and measurement complexity.21 AIP is an indirect indicator of small dense LDL levels. It has also been shown to be an important predictor of atherosclerosis and cardiovascular diseases and events.[9-10]

Previous rare studies have shown that the non-logarithmic triglyceride/HDL ratio is higher in patients with CSFP.[22-23] However, the logarithmic transformation of the triglyceride/HDL ratio is thought to better reflect atherogenic dyslipidemia.[8] In the present study, AIP was higher in patients with CSFP compared to the NCF group, despite similar demographic characteristics. The results of this study both support and extend the findings of previous limited studies that examined the relationship between AIP and CSFP. To the best of our knowledge, there were only two studies that investigated the relationship between AIP and NCFP. Afsin et al.[11] reported that patients with stable or unstable angina pectoris with CSFP had higher AIP compared to the NCF group. In a study conducted by Adalı et al.[12] on patients undergoing coronary angiography, AIP was approximately 2-folds higher in patients with CSFP than NCF patients. In these studies, confounding factors such as male gender, age, proportion of smokers, and presence of hypertension were higher in the CSFP group.[11-12] These confounding factors may have contributed in favor of CSFP, as they may be associated with a worse lipid profile.[24] The main difference between the present study and these studies is that the potential effects of these confounding factors were adjusted with a matched control group. On the other hand, consistent with the results of the studies mentioned above, AIP was identified as an independent predictor of CSFP. Current evidence supported that patients with CSFP may be predisposed to atherogenic dyslipidemia.

The AIP showed significant diagnostic performance in distinguishing patients with CSFP. The threshold value of AIP classified approximately 65% of patients with CSFP as true positive, while approximately 70% of individuals with NCF classified them as true negative. Afsin et al.11 reported the threshold value of AIP as 0.66 with 59% sensitivity and 73% specificity,

Table 1. Demographic and clinical findings in patients with and without slow coronary flow phenomenon.

Variables	CSFP group n = 110	NCF group n = 110	p
Demographic findings			
Gender, n (%)			
Female	33 (30.0)	34 (30.9)	0.884
Male	77 (70.0)	76 (69.1)	
Age, years	56.2 ± 8.3	55.6 ± 7.8	0.581
BMI, kg/m2	29.1 ± 3.8	28.9 ± 3.4	0.681
Smoking, n (%)	59 (53.6)	54 (49.1)	0.500
Diabetes mellitus, n (%)	40 (36.4)	33 (30.0)	0.316
Hypertension, n (%)	59 (53.6)	55 (50.0)	0.589
Dyslipidemia, n (%)	51 (46.4)	43 (39.1)	0.275
Clinical findings			
Systolic BP, mmHg	130.2 ± 24.3	128.1 ± 22.8	0.509
Diastolic BP, mmHg	78.3 ± 14.5	76.2 ± 13.3	0.264
LVEF, %	66.4 ± 7.8	67.8 ± 7.1	0.165
TFC, frame			
LAD	46.4 ± 2.5	22.4 ± 2.2	<0.001*
CX	34.5 ± 2.4	16.1 ± 2.1	<0.001*
RCA	31.3 ± 2.4	14.7 ± 2.2	<0.001*

Values are mean±SD or median (IQR) or number (%). * p<0.05 indicates statistical significance. Abbreviations: BMI: Body Mass Index; BP: Blood Pressure; CSFP: Slow Coronary Flow Phenomenon; Cx: Left Circumflex Coronary Artery; LAD: Left Anterior Coronary Artery; LVEF: Left Ventricular Ejection Fraction; NCF: Normal Coronary Flow; RCA: Right Coronary Artery; TFC: Thrombolysis in Myocardial Infarction Frame Count.

whereas Adalı et al.[12] reported 0.68 with 72% sensitivity and 42% specificity. Similar diagnostic performance has been reported in rare studies evaluating the non-logarithmic triglyceride/HDL ratio.[22-23] When the findings of this study are evaluated in the light of the existing literature, AIP may be an important indicator of CSFP. Threshold values in the 0.6-0.7 range of AIP in distinguishing CSFP are consistent across studies. Therefore, AIP before coronary angiography can be an important screening tool for estimating CSFP.

Another important finding of this study was that elevated neutrophil and platelet levels were an independent risk factor for CSFP in addition to AIP. Besides, higher monocyte levels were found in CSFP patients. This could be due to the relationship between atherogenic lipids and inflammation. It is postulated that HDL protects endothelial cells against the undesirable effects of LDL and exhibits both anti-inflammatory and antioxidant effects by preventing the oxidation of LDL molecules.[25] Atherogenic lipids, which cause low-grade inflammation in endothelial cells, may contribute to endothelial damage and acceleration of atherosclerosis.[5] Triglyceride-rich lipoproteins, which easily accumulate in the arterial wall, play a role in the accumulation of macrophages by causing endothelial damage and leukocyte activation.[19] Platelets play a key role in thrombotic events and blood rheology. The tendency to thrombogenicity causes slowing of blood flow and development of CSFP.[26] Some studies have indicated that platelet dysfunction associated with the development of CSFP.[27-28] In thromboinflammatory conditions, platelet-neutrophil interaction occurs and they regulate each other's functions.[29] An experimental study showed that activated polymorphonuclear neutrophils cause vasoconstriction and endothelial dysfunction in coronary arteries isolated from low-flow perfusion reperfused hearts.[30] Consistent with these findings, increased neutrophil and platelet levels were identified as an independent predictor of CSFP.

This study has some limitations. In addition to the small sample size, it had a single-center and

Table 2. Laboratory findings in patients with and without slow coronary flow phenomenon.

Variables	CSFP group n = 110	NCF group n = 110	p
FBG, mg/dL	92.1 ± 20.4	88.7 ± 19.4	0.207
Hemoglobin, g/dL	13.1 ± 2.3	13.5 ± 2.5	0.218
WBC, ×103/mm3	7.6 ± 2.3	7.3 ± 2.0	0.303
Neutrophil, ×103 µL	5.7 (3.4-8.1)	4.1 (2.3-6.0)	<0.001*
Lymphocyte, ×103 µL	2.1 ± 0.6	2.3 ± 0.7	0.024*
Platelets, ×103 µL	256.5 ± 56.2	203.6 ± 42.4	<0.001*
Monocyte, ×103 µL	0.6 ± 0.2	0.5 ± 0.1	<0.001*
RDW, %	13.8 ± 2.5	13.5 ± 1.8	0.308
Total cholesterol, mg/dL	200.4 ± 43.5	188.2 ± 37.1	0.026*
LDL, mg/dL	123.1 ± 28.0	115.2 ± 25.6	0.030*
HDL, mg/dL	39.1 ± 8.5	42.4 ± 9.2	0.006*
Triglyceride, mg/dL	145 (108-185)	122 (81-145)	0.010*
AIP	0.6 ± 0.2	0.4 ± 0.2	<0.001*

Values are mean±SD or median (IQR). * p<0.05 indicates statistical significance.
Abbreviations: AIP:Atherogenic Index of Plazma; CSFP: Slow Coronary Flow Phenomenon; FBG: Fasting Blood Glucose; HDL: High-Density Lipoprotein Cholesterol; LDL: Low-Density Lipoprotein Cholesterol; NCF: Normal Coronary Flow; RDW: Red Distribution Width; WBC: White Blood Cell.

Table 3. Independent predictors of slow coronary flow phenomenon.

Variables	Univariable				Multivariable			
	OR	95% CI		p	OR	95% CI		p
		lower	upper			lower	upper	
Neutrophil	1.21	1.09	1.35	<0.001*	1.15	1.02	1.29	0.011*
Lymphocyte	0.97	0.94	0.99	0.024*	-	-	-	-
Platelets	1.07	1.03	1.11	<0.001*	1.05	1.01	1.10	0.030*
Monocyte	1.03	1.01	1.08	<0.001*	-	-	-	-
Total cholesterol	1.06	1.01	1.12	0.026*	-	-	-	-
LDL	1.08	1.02	1.15	0.030*	-	-	-	-
AIP	17.0	4.91	58.97	<0.001*	15.33	4.11	57.18	<0.001*
					Nagelkerke R2: 0.326, p<0.001*			

Components of AIP were not included in the regression analysis. * p<0.05 indicates statistical significance.
Abbreviations: AIP: Atherogenic Index of Plazma; CI: Confidence Interval; LDL: Low-Density Lipoprotein Cholesterol; OR: Odds Ratio.

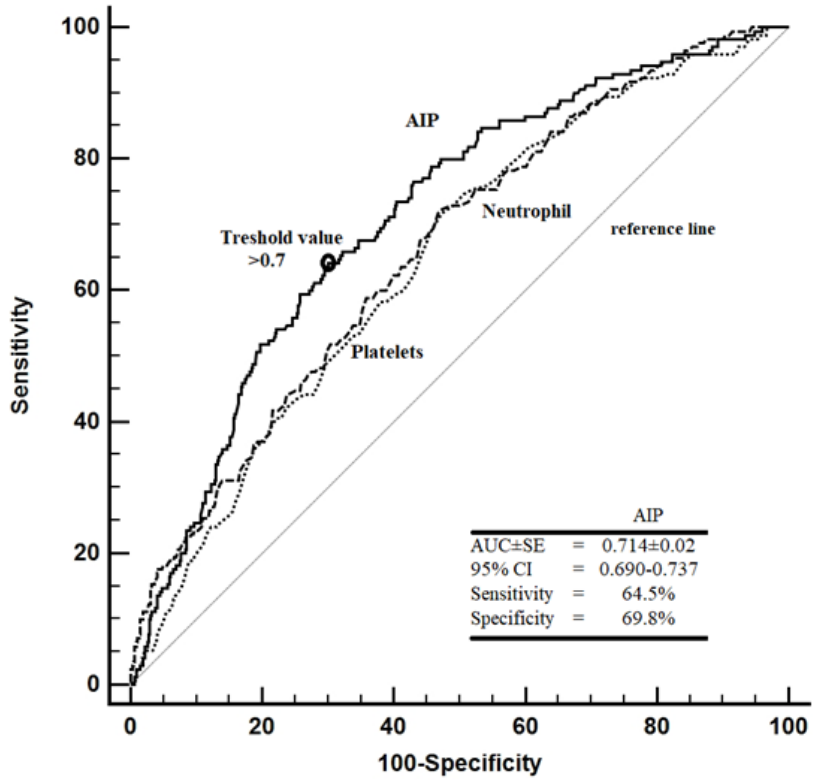

Figure 1. Diagnostic performance of the AIP in predicting slow coronary flow phenomenon.

retrospective design. The fact that the majority of patients were male may have contributed to the deterioration of homogeneity. Atherosclerosis-related inflammatory markers such as high-sensitivity C-reactive protein and interleukin-6 could not be studied due to the retrospective design. This limited the importance of the relationship between CSFP and inflammation. Apolipoprotein B and small dense LDL levels, which better reflect atherogenic dyslipidemia, could not be evaluated. In addition, endothelial dysfunction, which is involved in the pathology of CSFP, could not be evaluated by flow-mediated dilatation or pulse wave velocity.

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