ORIGINAL ARTICLE

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The Impact of Intravitreal Ranibizumab Treatment on Outer Retinal Layer Thickness in Neovascular Age-related Macular Degeneration

Neovasküler Yaşa Bağlı Maküla Dejenerasyonunda Intravitreal Ranibizumab Tedavisinin Dış Retinal Tabaka Kalınlığı Üzerine Etkisi

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ABSTRACT

Background: In this study, it was aimed to evaluate outer retinal layer thickness (ORLT) via spectral-domain optical coherence tomography (SD-OCT) in age-related macular degeneration (AMD) patients treated with intravitreal ranibizumab injection.

Materials and Methods: Patients with unilateral neovascular AMD who received intravitreal ranibizumab injection with a follow-up period of at least 24 months were retrospectively identified. The fellow eyes with dry AMD served as a control group. Data on best corrected visual acuity (BCVA) and OCT findings on ORLT were recorded at baseline, at 12- and 24-month follow-ups.

Results: Sixty-four eyes of 32 patients were included. The mean number of injections was 10.7 over a 24-month period. In the injection group, ORLT was 81.6±4.7 μm before the treatment, and decreased to 80.9±4.8 μm and 78.8±4.8 μm at 12- and 24-month follow-ups, respectively. In the control group, the same parameters were 81.9±4.4 μm, 81.6±4.4 μm and 80.8±4.3 μm, respectively. In both groups, a significant decline was noted in ORLT from baseline to 12- and 24-month follow-ups and from 12- to 24-month follow-ups ($p<0.001$, all values). While the injection and control groups were similar in terms of baseline ORLT, at the 24-month follow-up, ORLT was significantly lower in the injection group ($p=0.043$). The changes in BCVA were not significant from baseline to 12- and 24-month follow-ups in both groups ($p>0.05$, all values). A significant positive correlation was noted between the decrease in ORLT and the number of injections ($p<0.05$, all values).

Conclusion: ORLT was found to be decreased significantly in the natural course of AMD regardless of the subtype, whereas the decrease in ORLT was aggravated by ranibizumab injection in neovascular AMD eyes.

Keywords: Age-related macular degeneration, neovascular, OCT, outer retinal layer thickness, ranibizumab

ÖZ

Amaç: İntravitreal ranibizumab enjeksiyonu ile tedavi edilen yaşa bağlı maküla dejenerasyonu (YBMD) hastalarında dış retinal tabaka kalınlığının (DRTK) spektral-domain optik koherens tomografi (SD-OKT) ile değerlendirilmesi.

Gereç ve Yöntemler: Tek taraflı neovasküler YBMD nedeniyle intravitreal ranibizumab tedavisi almış en az 24 aylık takibi olan hastalar retrospektif olarak incelendi. Kuru tip YBMD olan diğer gözler kontrol grubu olarak kabul edildi. Başvuru anındaki, 12. aydaki ve 24. aydaki en iyi düzeltilmiş görme keskinlikleri (EİDGK) ve SD-OKT'deki DRTK değerleri kaydedildi.

Bulgular: Otuz iki hastanın 64 gözü dahil edildi. Yirmi dört aylık sürede ortalama enjeksiyon sayısı 10,7 idi. Enjeksiyon grubunda DRTK, tedaviden önce 81,6±4,7 μm iken, 12. ve 24. aylarda sırasıyla 80,9±4,8 μm'ye ve 78,8±4,8 μm'ye düştü. Aynı parametreler kontrol grubunda sırasıyla, 81,9±4,4 μm, 81,6±4,4 μm ve 80.8±4.3 μm idi. Her iki grupta da hem başlangıçtan 12. ve 24. ay kontrollere hem de 12. ay kontrolden 24. ay kontrole DRTK'de anlamlı düşüş vardı ($p<0,001$, tüm değerler). Başlangıçta DRTK açısından enjeksiyon ve kontrol grupları benzer olsa da 24 ayın sonunda DRTK enjeksiyon grubunda anlamlı derecede düşüktü ($p=0,043$). Her iki grupta da başlangıç, 12 ve 24. aylardaki EİDGK değişiklikleri anlamlı değildi ($p>0,05$, tüm değerler). DRTK azalma ve toplam enjeksiyon sayısı arasında pozitif anlamlı korelasyon izlendi ($p<0,05$, tüm değerler).

Sonuç: YBMD'nin alt tipinden bağımsız olarak hastalığın doğal seyrinde DRTK anlamlı olarak azalmaktadır. Bununla birlikte neovasküler YBMD'li gözlere uygulanan ranibizumab enjeksiyonu bu azalmayı şiddetlendirmektedir.

Anahtar Kelimeler: Dış retinal tabaka kalınlığı, neovasküler, OKT, yaşa bağlı maküla dejenerasyonu, ranibizumab

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Introduction

Neovascular age-related macular degeneration (AMD) is a progressive disease and a leading cause of permanent vision loss in the older age (1,2,3). The safety and efficacy of anti-vascular endothelial growth factor (VEGF) drugs in the management of neovascular AMD have consistently been reported worldwide, whereas because of limited information about anti-VEGF ocular pharmacokinetics, no standard practice exists regarding the administration schedules in clinical practice (4,5,6,7,8). Ranibizumab inactivates all VEGF-A isoforms, enabling anatomic and functional healing in choroidal neovascularization (CNV) in patients with neovascular AMD (5,6).

The introduction of spectral-domain optical coherence tomography (SD-OCT) to the practice of ophthalmology has provided high-resolution retinal images that help to collect detailed data on the quantitative assessment of each retinal layer for the investigation of diseases affecting specific retinal layers (9). The outer retinal layer, involving photoreceptors, retinal pigment epithelium (RPE), and Bruch's membrane, is considered likely to undergo degenerative changes related to the aging process. AMD-related changes are also characterized by an increase in thickness of the Bruch's membrane initially, progressing to a loss of RPE and photoreceptors in dry AMD, and the development of CNV in neovascular AMD (10,11).

Non-etheless, VEGF is a mediator with vital importance for retinal photoreceptors, Müller cells, and RPE as well as for the integrity of choriocapillaris (12,13). Inner retinal layers with a peripapillary nerve and retinal ganglion cell layer rather than outer retinal layer have become more extensively addressed by the segmentation studies in AMD patients with limited data on SD-OCT-based automatic segmentation analysis of outer retinal layer in relation to visual function in neovascular AMD patients (8,14,15,16,17). Hence, whether the VEGF suppression has potential age independent hazards on the outer retina remains an enigma.

This study was therefore designed to quantitatively evaluate outer retinal layer thickness (ORLT) via SD-OCT in neovascular AMD patients treated with intravitreal ranibizumab in comparison to fellow (untreated) eyes with dry AMD.

Material and Methods

Study Population

The examination records of patients with neovascular AMD who received intravitreal ranibizumab injection treatment at a tertiary-care ophthalmology clinic between January 2014 and December 2017 were retrospectively reviewed. Patients with neovascular AMD in one eye and dry AMD in the fellow eye were included in the study. They were treated with intravitreal ranibizumab with pro re nata (PRN) basis following three loading doses for subfoveal CNV and those with CNV lesions with borders not extending beyond the inner circle (r: 0.5-1.5 mm) of the ETDRS grid on OCT images. Fellow (untreated) eyes with dry AMD served as a control group. The exclusion criteria of the study included having inability to clearly detect retinal layers in OCT because of opacity, development of CNV in the control eye within 24 months, prior history of ocular surgery (except uncomplicated cataract surgery at least six months ago), comorbid diabetic retinopathy, retinal vein occlusion, ocular inflammation, glaucoma or optic nerve diseases and follow-up of less than 24 months.

This study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Ankara Training and Research Hospital (no. E-19-168). Patients gave written informed consent before the study procedures.

Assessments

All patients underwent complete ophthalmologic examination including measurement of best-corrected visual acuity (BCVA) by using Snellen chart and SD-OCT imaging at baseline and monthly visits as well as fundus fluorescein angiography before the first injection. BCVA was recorded as Snellen fractions and converted to equivalent a logarithm of the minimal angle of resolution (logMAR) values. Retreatment was given after three loading doses every four weeks on an as-required basis depending on predefined OCT (presence of subretinal and/or intraretinal fluid in monthly OCT imaging) and clinical criteria (hemorrhage in fundus examination). Data on patient demographics (age, gender), number of injections, ophthalmic examination findings, including BCVA (logMAR) and OCT findings on ORLT, were recorded both at baseline and at 12- and 24-month follow-ups and compared between neovascular AMD (injection group) and dry AMD (control group) eyes. The correlation of decrease in ORLT and the number of injections were also analyzed.

OCT Measurements

ORLT was measured with SD-OCT (Spectralis SD-OCT, Heidelberg, Germany) by two experienced examiners (BSG, MK). The technique for automated retinal segmentation of the SD-OCT device was performed to identify each retinal layer and quantify ORLT, from the external limiting membrane to the Bruch's membrane. Of the nine ETDRS macular areas (which include a central 0.5 mm circle, and

inner and outer rings measuring 0.5-1.5 mm and 1.5-3.0 mm in diameter, respectively), only measurements from outer ring were acquired, where disease pathology was usually least severe, and retinal layers were most easily distinguishable. An average of ORLT values obtained from superior, inferior, nasal, and temporal quadrants of the outer ring as automatically divided by the segmentation application of the SD-OCT device was evaluated (Figure 1).

Statistical Analysis

Statistical analysis was made using SPSS version 15.0. Data were expressed as mean ± standard deviation, minimum-maximum and percent (%) where appropriate. The normal distribution of the variables was tested using visual (histogram and probability plots) and analytical (Shapiro-Wilk tests) methods. The data showed an abnormal distribution; therefore, nonparametric tests were used for the analysis. The Mann-Whitney U test (for values between two independent groups) and the Friedman test (for values among three dependent groups) were used to analyze numerical variables. If there was a significant difference among three or more independent groups, the Bonferroni correction was applied in post-hoc binary comparisons. Correlation analysis was performed using the Spearman correlation analysis. We considered $p<0.05$ as statistically significant.

Results

Sixty-four eyes of 32 patients were included in the study. The mean age of patients at the initiation of treatment

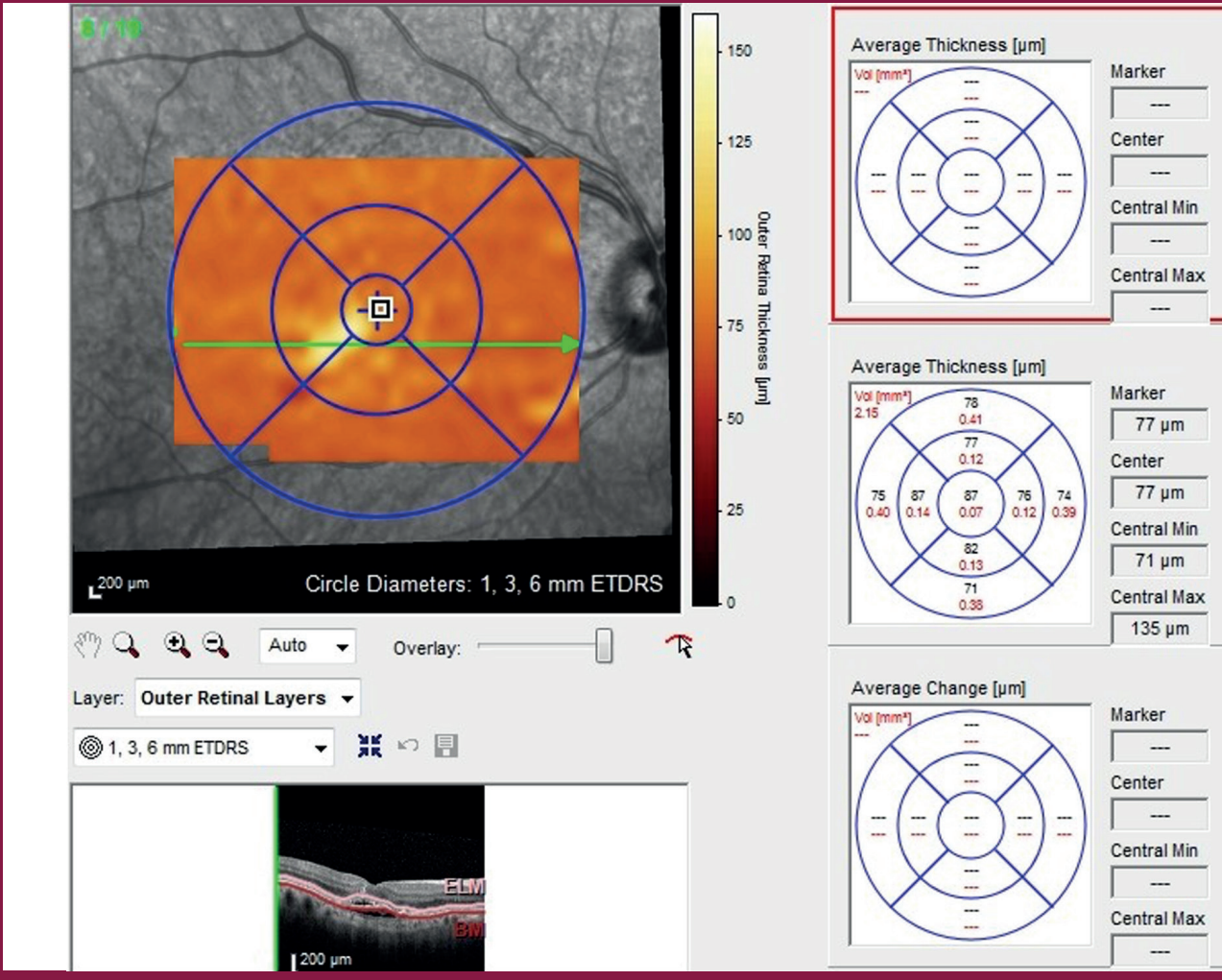

Figure 1. Representation of automated retinal layer segmentation and thickness measurements obtained by spectral-domain optical coherence tomography, regarding average ORLT values obtained from superior, inferior, nasal and temporal quadrants of outer ring as automatically divided by the segmentation application of the SD-OCT device
ORL: Outer retinal layer, SD-OCT: Spectral-domain optical coherence tomography

was 68.4±2.1 years (range: 57 to 78). There were 14 males (43.7%) and 18 females (56.3%). The mean number of injections was 10.7±3.5 (range: 7 to 17) over a 24 month period. In the injection group, the mean ORLT was measured as 81.6±4.7 µm (range: 75 to 93) before the treatment, and decreased to 80.9±4.8 µm (range: 75 to 92) and 78.8±4.8 µm (range: 72 to 90) at 12 and 24-month follow-up respectively. In the dry AMD (control) group, the same parameters were 81.9±4.4 µm (range: 75 to 93), 81.6±4.4 µm (range: 75 to 92), and 80.8±4.3 µm (range: 74 to 91), respectively. In both injection and control groups, a significant decline was noted in ORLT from baseline to 12 and 24 months follow-up and from 12 to 24 months follow-up (p<0.001, all values) (Table 1). While the injection and control groups were similar in terms of baseline ORLT, at the 24-month of follow up, ORLT was significantly lower in the injection group (p=0.043) (Table 1).

Considering BCVA, in the injection group, the mean BCVA was found to be improved at each annual follow-up compared to that of baseline, but not significantly (p>0.05). Also, the changes in BCVA were not statistically significant in the control group from baseline to 12- and 24-months follow-up (p>0.05) (Table 1).

A significant positive correlation was noted between the decrease in ORLT and the number of injections from baseline to 12- month (Figure 2a) and 24-month follow-up (Figure 2b) and from 12-month to 24-month follow-up (Figure 2c), (r=0.472, p=0.006; r=0.632, p<0.001; r=0.376, p=0.034, respectively) in the injection group.

Discussion

VEGF has been considered as an important mediator for the development of CNV in animal studies (18). Intravitreal

Table 1. ORL thickness and BCVA in injection and control eyes

		Baseline	12th month	24th month	p[a]
		mean ± SD (min-max)	mean ± SD (min-max)	mean ± SD (min-max)	
ORL (µ)	Injection	81.6±4.7 (75-93)	80.9±4.8 (75-94)	78.8±4.8 (72-92)[0,12]	<0.001**
	Control	81.9±4.4 (76-92)	81.6±4.4 (75-91)	80.8±4.3 (74-91)[0,12]	<0.001**
	p[b]	0.691	0.462	0.043	
GK (LogMAR)	Injection	0.61±0.32 (0.1-1.0)	0.59±0.35 (0-1.0)	0.58±0.38 (0-1.0)	0.308
	Control	0.16±0.13 (0-0.5)	0.17±0.13 (0-0.5)	0.17±0.13 (0-0.5)	0.577
	p[b]	<0.001**	<0.001**	<0.001**	

SD: Standard deviation, [a]: Friedman test, [b]: Mann-Whitney U test, 0: There was a significant difference in post-hoc binary comparison with "baseline", 12: There was a significant difference in post-hoc binary comparison with "12th month" ORL: Outer retinal layer, BCVA: Best corrected visual acuity, LogMAR: Logarithm of the minimal angle of resolution

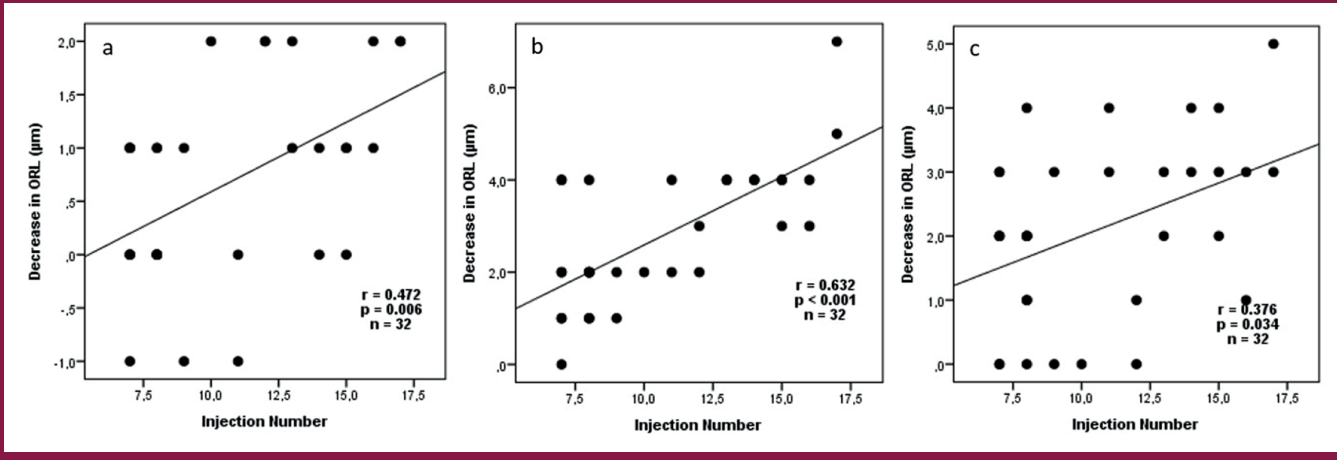

Figure 2. Scattered diagrams showing a positive correlation between decrease in ORLT and the number of injections from baseline to 12-month follow-up (r=0.472, p=0.006) (a), baseline to 24-month follow-up (r=0.632, p<0.001) (b), and from 12-month to 24-month follow-up (r=0.376, p=0.034) (c)
ORLT: Outer retinal layer thickness

usage of anti-VEGF drugs increases vision by reducing leakage from CNV, but repeated injections are required to maintain the effect. When the treatment regimen is applied steadily at regular intervals, the best visual acuity is achieved, while the risk of unnecessary injections into the dry lesion arises. In the PRN regimen, injection is made during monthly controls depending on whether there is any fluid present in OCT (19). In any kind of treatment regimens, anti-VEGF therapy should be continued for a long time related to the natural course of the disease. Continuous inhibition of VEGF with anti-VEGF therapy can reduce the VEGF level, which is also necessary for ocular homeostasis thereby causes RPE and choriocapillaris atrophy (20,21).

In the SEVEN-UP study that reports seven-year results of intravitreal ranibizumab injection in the treatment of CNV secondary to AMD, 98% of the eyes developed macular atrophy (22). In the two-year results of the Comparison of Age-Related Macular Degeneration Treatment Trials (CATT) study comparing ranibizumab and another anti-VEGF drug, i.e., bevacizumab, it was reported that more geographic atrophy was observed in patients receiving monthly treatment for both agents compared to the PRN group. It was also reported that VEGF was important to keep normal functioning of the retina and the integrity of the choriocapillaris via RPE, and its blockage might cause the development and progression of geographic atrophy (23). Five-year results of the CATT study showed that there was no statistical difference between monthly treatment and PRN treatment regimens in terms of the risk of developing geographic atrophy (24). According to clinical studies, since there is an increase in the frequency of geographic atrophy in eyes treated with anti-VEGF therapy, whether this condition develops in association with the anti-VEGF therapy or results from the natural course of the disease is controversial.

Of experimental studies to demonstrate the retinal toxicity of anti-VEGF therapy are quite controversial either. In an animal study with mice, systemic administration of the adenoviral vector expressing VEGFR1 was shown to cause photoreceptor degeneration, and it was emphasized that VEGF must have been vital for photoreceptors and Müller cells (12). However, various publications show that VEGF suppression does not negatively affect photoreceptors (13,25).

So, is it possible to show clinically the effects of anti-VEGF medications on retina and RPE? The segmentation feature in the new generation OCTs could be used to assess the progression of specific retinal disorder by quantitatively measuring the thickness of the retinal layers.

OCT-segmentation studies enable to report thickness changes in inner retinal layers associated with the treatment of neovascular AMD with ranibizumab (14,15,16,17). Moreover, in an OCT segmentation study that investigated the impact of aging on the outer retinal layer and choroid, it was shown that the RPE and photoreceptor layers and the choroidal thickness decreased with increasing age (26). Another study reported that patients with dry AMD in one eye had more thinning in the RPE-photoreceptor layer thickness in the healthy eye compared to the normal population of the same age group (27).

In a study evaluating the effect of intravitreal ranibizumab treatment of neovascular AMD on all retinal layers, it was reported that there was a significant thinning in the inner retina layers after one year of treatment, and a significant decrease in total outer retinal layer and RPE thickness was restricted to occur for the first three months (14). However, the lack of a control group in the relevant study cannot exclude the effect of the natural course of the disease in this thinning. The current study revealed a significant decrease in ORLT in neovascular AMD patients treated with ranibizumab when compared to both pretreatment levels and corresponding outer retinal layer values in fellow eyes with dry AMD. Notably, a decrease was noted in ORLT along with an increase in the number of ranibizumab injections in eyes with neovascular AMD. In the presence of CNV, the evaluation of the outer retinal layers using the OCT segmentation method is difficult because the lesion often has complex configuration. In the current study, measurements from the outer ring were acquired enabling data on the outer retinal layer from quadrants outside the CNV lesion, where disease pathology was usually least severe and retinal layers were most easily distinguishable, increasing the reliability of measurements. Hence, our findings suggest a decrease in ORLT in both injection (neovascular AMD) and control (dry AMD) eyes during follow-up, whereas there is an association of intravitreal ranibizumab injection with further reduction in ORLT in eyes with AMD as compared to outer retinal layer changes in the fellow (untreated) eyes with dry AMD.

Our findings revealed no significant change in BCVA at the end of the two-year ranibizumab injection treatment. The reason for this insignificant increase in visual acuity may be due to the regimen applied. In the HORIZON study, it was reported that the increase in vision which was achieved with monthly ranibizumab injections for two years decelerated upon lowering the injection frequency (6). Another reason for the insignificant increase in visual acuity may be due to the fact that all of the lesions included in this study were located subfoveally. However, besides the poorer visual acuity at baseline in eyes with neovascular AMD versus dry AMD, no significant change was noted in

visual acuity under ranibizumab therapy in neovascular AMD eyes in our cohort, despite the correlation of frequency of injections with a decrease in ORLT. Nonetheless, our findings support the likelihood of considering additional morphologic characteristics of CNV lesions on OCT to improve the observed correlations of retinal changes with visual function (28).

The major strength of the current study is the inclusion of fellow eyes as for the control group to recognize the direct effects of ranibizumab injection on ORLT and the use of OCT data from the areas outside of the CNV lesion in measuring ORLT improved the reliability of measurements. However, certain limitations of this study should be considered. First, the relatively low sample size might prevent us from achieving statistical significance concerning the visual acuity changes and limit the generalizability of our results. Second, the changes in total retinal thickness were not evaluated in the study. Therefore, it is not possible to assess whether the resolution of edema may be more responsible for the thinning rather that "drug induced atrophy".

In conclusion, in the current study, ORLT was found to be decreased significantly in the natural course of AMD regardless of the subtype, whereas the decrease in ORLT was aggravated by ranibizumab injection in neovascular AMD eyes.

Study Limitations

The relatively low sample size might prevent us from achieving statistical significance concerning the visual acuity changes and limit the generalizability of our results. And also, the changes in total retinal thickness were not evaluated in the study. Therefore, it is not possible to assess whether the resolution of edema may be more responsible for the thinning rather that drug induced atrophy.

Conclusion

In the current study ORLT was found to be decreased significantly in the natural course of AMD regardless of the subtype, whereas the decrease in ORLT was aggravated by ranibizumab injection in neovascular AMD eyes.

Ethics

Ethics Committee Approval: Ethics committee approval was received from Ankara Training and Research Hospital (no: E-19-168).

Informed Consent: Patients gave written informed consent before the study procedures.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.Ş.G., M.K., Concept: B.Ş.G., Design: B.Ş.G., Data Collection or Processing: M.K., Analysis or Interpretation: B.Ş.G., Literature Search: B.Ş.G., Writing: B.Ş.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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The Relationship Between Monocyte Level on Admission and in Hospital Mortality in ST-elevation Myocardial Infarction Patients

ST-yükselmeli Miyokard İnfaktisü Hastalarında Başvuru Monosit Seviyesinin Hastane İçi Mortalite ile İlişkisi

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ABSTRACT

Background: Inflammation plays a key part in atherosclerotic processes. For inflammation balance, monocytes mission is essential. The importance of regulated inflammations has been known in ST-segment elevation patients for a long time. Therefore, we investigated the relationship between monocyte level on admission and in-hospital mortality in ST-elevation myocardial infarction (STEMI) patients.

Materials and Methods: A total of 2.341 serial patients in STEMI treated by primary percutaneous coronary intervention in a tertiary heart center between December-2008 and October-2014 were enrolled and categorized into two groups as low and high monocyte groups.

Results: There were 1.594 (68.0%) patients in the low monocyte (≤0.7x10^3/μL) group and 747 (31.9%) patients in the high monocyte (>0.7x10^3/μL) group. High monocyte group had larger size infarct area so impaired left ventricular ejection fraction. In multivariate analysis, monocyte count remained as an independent factor for in-hospital deaths (odds ratio: 2.63, 95% confidence interval: 1.07-6.47; p=0.040).

Conclusion: The current study demonstrated that admission monocyte level was independently related to in-hospital death. Therefore, admission monocyte count might be a useful tool in early risk scoring for STEMI patients.

Keywords: Primary PCI, ST-segment elevation myocardial infarction, monocyte level

ÖZ

Amaç: Enflamasyon, aterosklerozun patogenezinde anahtar rol oynar ve monositler enflamasyonun ana düzenleyicilerinden biridir. ST yükselmeli miyokard enfarktüsü (STyME) hastalarında iyi dengelenmiş enflamasyonun önemi uzun yıllardır tanımlanmıştır. Bunun sonucu olarak, STyME ile hastaneye başvuran hastaların başvuru monosit değeri ile hastane içi ölüm arasındaki ilişkiyi araştırdık.

Gereç ve Yöntemler: Aralık 2008-Ekim 2014 tarihleri arasında üçüncü basamak bir kalp merkezine başvuran ve primer perkütan girişim uygulanmış 2,341 STyME hastası kayıt altına alınıp yüksek ve düşük monosit sayısına göre iki gruba ayrıldı.

Bulgular: Düşük monosit (≤0,7x10^3/μL) grubunda 1,594 (%68,1) ve yüksek monosit(>0,7x10^3/μL) grubunda 747 (%31,9) hasta bulunmaktaydı. Yüksek monosit grubunda daha fazla infarkt alanı ve dolayısıyla daha düşük ejeksiyon fraksiyonu tespit edildi. Yapılan çoklu değişkenli analizde monosit değeri ile hastane içi ölüm arasında bağımsız ilişki bulundu (odds oranı: 2,63, %95 güven aralığı: 1,07-6,47; p=0,040).

Sonuç: Bu çalışmada başvuru monosit değeri ile hastane içi ölüm arasında bağımsız ilişki gösterildi. Sonuç olarak başvuru monosit değeri, STyME hastalarının erken risk skorlamasında kullanışlı olabilir.

Anahtar Kelimeler: Primer PKG, ST-yükselmeli miyokard infarktüsü, monosit sayısı

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Introduction

ST-segment elevation myocardial infarction (STEMI) is the most important type of acute coronary syndromes (1). Inflammation and immunological processes play an important role particularly in the development and pathogenesis of acute coronary syndromes: STEMI, non-STEMI, and unstable angina pectoris (2). Immune system cells like lymphocytes, neutrophils and monocytes are significantly associated with atherosclerotic processes, endothelial dysfunction, and ventricular remodeling in patients with STEMI. Monocytes are a part of innate immunity and are involved in reparative processes. Recent studies revealed that plateletcrit (3) ratio of neutrophil-to-lymphocyte (NLR) (4,5), ratio of lymphocyte-to-monocyte (6,7) and ratio of eosinophil-to-monocyte (EMR) (8) were directly related to severity, mortality and clinical outcomes in STEMI and coronary artery diseases. Here, our purpose was to determine the relationship between the monocyte level on admission and in-hospital mortality in STEMI patients.

Materials and Methods

Study Population

We enrolled a total of 2.341 consecutive patients with STEMI, who were treated with primary percutaneous coronary intervention (PPCI) and whose chest pain duration was lower than 24 hours, from December, 2008 to October, 2014 in a tertiary heart center. STEMI was described to be typical angina pectoris longer than 20 minutes, with new onset of left bundle branch block or ST elevation >1 mm at minimum two contiguous leads on the electrocardiogram and >2 fold up in serum cardiac markers, specifically troponin (9). Patients having cardiogenic shock and active infections and being treated with thrombolytic therapy were excluded. We divided all patients into two groups. The first group was named as the low monocyte group, which meant monocyte count was equal or lower than 0.7x10^3/uL, and the other group was named as the high monocyte group, which meant monocyte count was higher than 0.7x10^3/uL. Ethics committee approval and patient consent were obtained for the study.

Coronary Angiography

All patients received antiaggregant therapy with clopidogrel (600 mg) and aspirin (300 mg) prior to PPCI. Also, during the procedure, patients were treated with intravenous bolus of unfractionated heparin at a dose of 70-100 U/kg of body weight. The statin, beta-blocker agents which had no contraindications were ordered for the patients. Stenting was the main interventional treatment in our center. Radial or femoral approach, using of balloon for predilatation or postdilatation, stent type, and all equipment were left to the operator's decision.

Data Collection

All patients' age, gender, current smoking status, comorbidities such as hypertension, diabetes, hyperlipidemia, and chronic renal failure were recorded. Also, past medical histories including myocardial infarction, PCI, or coronary artery bypass graft stories of patients were recorded. Blood samples were taken prior to aspirin and clopidogrel administration for the measurement of laboratory parameters. Blood samples were taken into standard Ethylenediaminetetraacetic acid containing tubes and evaluated by an automated blood cell counter (LH 780; Beckman co.). All biochemical parameters were noted. Echocardiographic assessment was performed before PPCI with GE ViVidE7 ultrasound machine by using the Simpson's method. Left ventricular ejection fraction was measured in apical four chamber view. We also recorded the patients' angiographic characteristics.

Statistical Analysis

All continuous variables were expressed as mean±standard deviation. The Kolmogorov-Smirnov test was used for testing normality. The independent sample t-test was used for continuous variables displaying normal distributions. The Mann-Whitney U test was performed for continuous variables with skewed distributions. Categorical variables were expressed as number and percentages and the Pearson's chi-square test was employed for the evaluation of differences. For multivariable analysis, hierarchical logistic regression model was used. The odds ratio (OR) demonstrated the relative risk of death in the groups. Confounders of multivariate analysis were the predictors of in-hospital mortality. The p-value of <0.05 was considered statistically significant, and 95% confidence intervals (CI) were presented for all hazard and ORs. Statistical Package for Social Sciences software, version 15.0 (SPSS; IBM, Armonk, New York, USA) was used for analyses.

Results

A total of 2.341 patients were included in our study and treated with PPCI, and stent implantation was technically successful. The patients were classified into two groups: low monocyte and high monocyte groups, as shown in Table 1. The mean age of patients was 57 years, and 1.989

Table 1. Baseline and laboratory characteristics of patients

Variables	Low monocyte (≤0.7x10^3/μL) (n=1.594)	High monocyte (>0.7x10^3/μL) (n=747)	p
Age, y	57.82±11.6	55.44±11.87	<0.001
Male, n	1323 (56.5%)	666 (28.4%)	<0.001
Diabetes, n	410 (17.5%)	183 (7.8%)	0.542
Hypertension, n	477 (20.3%)	201 (8.5%)	0.146
Current smoking, n	607 (25.9%)	343 (14.65%)	<0.001
Hyperlipidemia, n	434 (18.53%)	196 (8.3%)	0.638
Stroke, n	24 (1.0%)	8 (0.3%)	0.413
Previous CABG, n	40 (1.7%)	28 (1.1%)	0.095
Previous PCI, n	207 (8.8%)	94 (4.0%)	0.874
In-hospital mortality, n	30 (1.2%)	24 (1.0%)	0.046
Hemoglobin, g/L	13.5±1.7	13.9±1.9	<0.001
White blood cell count, 10^9/L	11.1±4.0	14.5±4.6	<0.001
Platelet count, 10^9/L	233±67	249±70	<0.001
Monocyte count, 10^9/L	0.48±0.1	1.02±0.6	<0.001
Serum creatinine, mg/dL	0.91±0.3	0.94±0.3	0.014
Total cholesterol, mmol/L	184±51	178±45	0.018
Triglyceride, mmol/L	156±102	163±103	0.141
HDL, mmol/L	38.2±10.2	36.8±10.2	0.004
LDL, mmol/L	114.6±39.1	109.0±36.6	0.003
Glucose, mg/dL	159.2±80.1	157.9±82.0	0.712
CKMB, U/L	156±144	177±171	0.004
Troponin, ng/mL	49.0±4.5	49.1±5.7	0.915

CABG: Coronary artery bypass graft, CKMB: Creatine kinase-myocardial band, HDL: High density lipoprotein, LDL: Low density lipoprotein, PCI: Percutaneous coronary intervention

Table 2. Angiographic characteristics of patients

Variables	Low monocyte (≤0.7x10^3/μL) (n=1594)	High monocyte (>0.7x10^3/μL) (n=747)	p
Infarct-related coronary artery LAD LCx RCA	 681 (29.0%) 230 (9.8%) 649 (27.7%)	 339 (14.4%) 127 (5.4%) 261 (11.1%)	0.031
Number of used stents	1345 (57.4%)	607 (25.4%)	0.074
Contrast agent use, mL	244±85	250±87	0.132
Multivessel disease	301 (12.8%)	157 (6.7%)	0.214
Tirofiban use	759 (32.4%)	387 (16.5%)	0.051

LAD: Left anterior descending artery, LCx: Left circumflex artery, RCA: Right coronary artery

patients were male, 352 patients were female. We listed the demographic, laboratory and clinical characteristics in Table 1. We also analyzed angiographic characteristics and demonstrated them in Table 2. We searched the variables' effects on in-hospital mortality and reached the result that high monocyte count, high LDL level, old age (>65 age), male gender, diabetes, high serum creatine level (>2 gr/dL), low left ventricular EF (<%50) and high CK-MB directly were associated with in-hospital death (Table 3). Multivariate analysis determined that the independent parameters of in-hospital mortality, high monocyte count (OR: 2.63; 95% CI: 1.07-6.47; p= 0.03), older age, diabetes, high LDL level, low left ventricular EF and high CK-MB level were independently related to in-hospital mortality (Table 4).

Table 3. Univariate analysis

Variables	Odds ratio	95% confidence interval	p
Male	0.45	0.24-0.83	0.013
Age	1.07	1.05-1.10	<0.001
Creatinine	9.20	5.52-15.34	<0.001
Diabetes	3.82	2.21-6.59	<0.001
Hypertension	1.70	0.98-2.96	0.051
Current smoking	0.66	0.37-1.19	0.174
Hyperlipidemia	0.21	0.07-0.59	0.003
Chronic renal failure	9.33	3.95-22.04	<0.001
Peak CKMB	1.00	1.00-1.00	<0.001
Previous PCI	0.84	0.35-1.98	0.696
LVEF	0.91	0.89-0.93	<0.001
White blood cell count	1.11	1.06-1.16	<0.001
Hemoglobin count	0.83	0.72-0.96	0.012
Platelet count	1.00	0.99-1.00	0.926
HDL	0.95	0.91-1.00	0.053
LDL	0.96	0.95-0.98	<0.001
Peak troponin	0.98	0.94-1.03	0.513
High monocyte	1.73	1.00-2.98	0.041

CKMB: Creatine kinase-myocardial band, HDL: High density lipoprotein, LDL: Low density lipoprotein, LVEF: Left ventricular ejection fraction, PCI: Percutaneous coronary interventio

Table 4. Multivariate analysis

Variables	Odds ratio	95% confidence interval	P
Age	1.07	1.03-1.11	<0.001
Diabetes	2.54	1.04-6.18	0.042
LVEF	0.95	0.91-1.04	0.051
LDL	0.97	0.95-0.98	<0.001
Peak CKMB	1.00	1.00-1.00	<0.001
High monocyte	2.63	1.07-6.47	0.031

CKMB: Creatine kinase-myocardial band, LDL: Low density lipoprotein, LVEF: Left venricular ejection fraction

Discussion

Monocytes have an important role in atherogenesis and take part in inflammation (10). Monocytes consist of heterogeneous cell population and contain several surface expressions like CD14 and CD16 (11). CD16+ monocytes entitled proinflammatory cells and answer on inflammation (12). Also, monocytes have been effective in lots of systemic inflammatory diseases like rheumatoid arthritis and systemic lupus erythematosus. In atherosclerotic way, monocytes are related to stable and unstable plaque (13). In more than 900 stable CAD patients, it was shown that high monocyte count was strongly related to adverse cardiovascular events. (14).

Monocytes are modifiable cells enhancing their interaction with endothelial cells and myocardial cells. Increased expression of MAC-1 receptor leads to robust monocyte adhesion to the endothelial tissue (15). Intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and L-selectin levels also increase in beginning part of acute coronary syndromes (16). Acute coronary syndromes are related to upregulation of some receptors like fibronectin receptor VLA-5, the mission of which is migration to tissue and very important part of cardiac extracellular matrix proteins (17). In acute coronary syndrome pathogenesis, monocyte chemoattractant protein (MCP-1) and macrophage colony stimulating factor (M-CSF) play a role in monocyte collection into the infarct zone, differentiation of monocytes to macrophages in the infarct area (18). MCP-1 (gene name *CCL2*, receptor gene name *CCR2*) is the most important chemokine that orchestrates to the macrophages' roles. Blood flow reduction stimulates MCP-positive macrophage infiltration of injured myocardium. (19). Circulating monocytes produce high plasma levels of MCP-1 in acute coronary syndromes, and this situation leads to neovascularization, so much macrophage collection and accumulation of myofibroblasts that affects left ventricular remodeling (20).

Our study showed an important relationship between high blood level of monocytes and in-hospital mortality in STEMI patients who were treated with PPCI. In previous studies, researchers have collected lots of information to show an association between inflammatory cells and development processes of atherogenesis. For remembering, monocytes play a key role in atherosclerosis firstly (21). Endothelial dysfunction leads to the accumulation of inflammatory cells in the endothelium of arterial wall (22). Monocytes which migrate to the tissue get the name of macrophage (23). Macrophages release some factors like interleukin-6 (IL-6), tumor necrosis factor alpha for more inflammation (24). Lots of clinical studies have shown that advanced monocyte level is associated with high inflammation, large infarct areas, and left ventricular dysfunction (25). Inhibiting monocytes collecting to infarcted myocardium tissue might prevent loss of left ventricular ejection fraction (26). Some studies focused on eosinophil to monocyte ratio (27) and neutrophil to lymphocyte ratio (28), and they showed again inflammation strongly related to pathogenesis of atherosclerosis.

In present study, we clearly showed that high monocyte level in patients with STEMI was related to larger infarct size, lower left ventricular ejection fraction and higher in-

hospital mortality. Therefore, monocyte level on admission may be useful for the prediction of early risk score for in-hospital mortality.

Study Limitations

Our study has several limitations. First, it was a single center and retrospective study. Second, we excluded STEMI patients having cardiogenic shock on admission, active infection and being treated with thrombolytic therapy. We need larger patient population and prospective and randomized clinical studies for suggestion of our findings.

Conclusion

We showed that higher monocyte level on admission was related to higher risk for in-hospital mortality. Monocyte count could be a simple and useful biomarker for risk stratification in STEMI patients.

Ethics

Ethics Committee Approval: Ethics committee approval and patient consent were obtained for the study (protocol number: 2800l928-501.07.0l-29.07.15).

Informed Consent: Patient consent were obtained for the study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.H., Concept: R.H., Design: M.K., Data Collection or Processing: R.H., Analysis or Interpretation: M.K., Literature Search: Ş.Ü.D., Writing: R.H.

Conflict of Interest: No conflict of interest was declared by the authors.

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The Expression Levels of MicroRNA-31, MicroRNA-125a and MicroRNA-125b in Chronic Rhinosinusitis with Nasal Polyp

Nazal Polipli Kronik Sinüzitte MikroRNA-31, MikroRNA-125a ve MikroRNA-125b Seviyeleri

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ABSTRACT

Background: Nasal polyposis is a disease with chronic inflammation of the sinonasal mucosa. Expression of micro-RNAs is altered in many diseases. The goal of this study is to evaluate the expression levels of inflammation associated micro-RNAs in chronic rhinosinusitis with nasal polyposis and to show the correlation between nasal polyps and micro-RNAs.

Materials and Methods: This retrospective study was designed in patients who underwent endoscopic sinus surgery between January 2006 and June 2015. One hundred and ten patients who underwent endoscopic sinus surgery in our clinic were included in the study. Micro-RNA31, micro-RNA125a, and micro-RNA125b were evaluated using real time quantitative reverse transcription polymerase chain reaction in adjacent normal nasal mucosa of nasal polyp and nasal polyp samples of the patients with chronic rhinosinusitis with nasal polyposis.

Results: Only 40 patients met the inclusion criteria. The mean micro-RNA levels of micro-RNA-125b, micro-RNA-125a, and micro-RNA-31were found to be elevated in nasal polyp tissues compared to adjacent normal nasal mucosa, but there was no statistically significant difference in the expression levels of miRNA-125b, miRNA-31 and miRNA-125a between normal tissue and nasal polyp.

Conclusion: We could not find any results indicating that miRNA-125b. miRNA31 and miRNA-125a would be effective in the pathogenesis of nasal polyp.

Keywords: MicroRNA, chronic rhinosinusitis, nasal polyp, inflammation

ÖZ

Amaç: Kronik sinüzite eşlik eden nazal polipozis sinonazal mukozanın enflamasyonu ile karakterize bir sendromdur. MikroRNA'ların düzensiz ifadesi birçok hastalıkta gösterilmiştir. Amacımız kronik sinüzite eşlik eden nazal polipozis hastalarında üç farklı mikroRNA'nın ekspresyon düzeylerini değerlendirmek ve mikroRNA'lar ile nazal polipozis arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntemler: Çalışma retrospektif olarak yürütüldü ve Ocak 2006-Haziran 2015 tarihleri arasında kronik sinüzite eşlik eden nazal polipozis nedeniyle endoskopik sinüs cerrahisi uygulanan 110 hasta çalışmaya dahil edildi. Bu hastaların nazal polip dokuları ve bitişiğindeki sağlam nazal mukoza dokularında enflamasyon ile yakından ilgili olduğu bilinen üç farklı mikroRNA (mikroRNA31, mikroRNA125a, mikroRNA125b) düzeyleri gerçek zamanlı kantitatif revers transkripsiyon polimeraz zincir reaksiyonu kullanılarak analiz edildi.

Bulgular: Çalışmaya dahil edilen 110 hasta arasından sadece 40 hasta bu çalışma için uygun bulundu. mikroRNA-31, mikroRNA -125a ve mikroRNA-125b'nin ortalama mikroRNA seviyelerinin, normal nazal mukozaya kıyasla nazal polip dokularında arttığı bulundu, ancak fark istatiksel olarak anlamlı değildi.

Sonuç: Kronik sinüzite eşik eden nazal polipozis patgogenezinde mikroRNA-31, mikroRNA-125a ve mikroRNA-125b'nin etkili olabileceğini gösteren herhangi bir sonuç bulunmadı.

Anahtar Kelimeler: MikroRNA, kronik sinüzit, nazal polip, enflamasyon

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Introduction

Chronic rhinosinusitis (CRS) is an important disease that severely affects patients' quality of life. It is a sinus disease of nasal cavity and paranasal sinuses in which the sinonasal mucosa is severely and chronically inflamed. CRS is basically divided into two sub-types based on the absence or presence of nasal polyps (NPs): CRS without NPs (CRSsNP) and CRS with NPs (CRSwNP) (1). CRSsNP and CRSwNP have different histologic and immunologic features. Inflammatory cells, particularly B cells and plasma cells are significantly higher in CRSwNP compared to CRSsNP, while the numbers of mucosal glands are higher in CRSsNP compared to CRSwNP (1,2,3,4,5). Tissue remodeling is a part of wound healing and it is a dynamic process involving a structural reconstruction of the tissue (6).

Micro-RNAs (miRNAs) are relatively new defined, small, measuring approximately ~22 nucleotides in length, noncoding single-stranded RNAs that comprise a new class of gene regulators. miRNAs act primarily as gene expression suppressors (7,8). miRNAs regulate nearly half of the *protein-coding* genes. These genes bind to the 3' UTR of target mRNA and genes show their effects by inhibition of the translation of target mRNA using degradation or silencing pathways (9,10,11). The first miRNAs (lin-4 and let-7) were discovered in Caenorhabditis elegans, from that time researcher have reported over 2000 miRNAs in the human genome (12,13,14). Impaired expression of miRNAs has been shown in many diseases, especially in neoplasms and immunologic and inflammatory diseases, for instance allergic asthma, allergic rhinitis, and atopic dermatitis (7,8). Studies on miRNA-31 reveal that it shows different expression levels in different tumors. The *miRNA-31* gene is located on chromosome band 9p21.3. It encodes the cell cycle inhibitor proteins p15 and p16 (15). microRNA-125 family (miRNA-125) consists of miRNA- 125a and miRNA-125b. They regulate cell differentiation, proliferation, and apoptosis in cells. miRNA-125a and miRNA-125b may lead to diseases such as cancer and autoimmune diseases (14).

The goal of this study is to evaluate the expression levels of three different microRNAs (miRNA31, miRNA125a, miRNA125b) in CRSwNP.

Material and Method

Patients and Sample Collection

The study was conducted as a retrospective study. Tissue samples of 40 (n=40) patients who met the inclusion criteria among 110 patients undergoing endcoscopic sinus surgery between 2006 and 2015 were included in the study. The patients who underwent endoscopic sinus surgery due to bilateral CRSwNP were included in the study. Ethics committee approval of the study was obtained from of GATA Haydarpaşa Numune Training and Research Hospital (no: 1491-128-13/1539 date: 20/01/2014). Written informed consent was obtained from the patients.

Exclusion criteria included the presence of systemic diseases, fungal sinusitis, congenital mucociliary problems, antrochoanal polyps and cystic fibrosis. Patient characteristics are presented in Table 1.

Selection of miRNAs

The miRNAs included in the study were determined as a result of the literature review (11,15,16). Three different miRNAs (miRNA31, miRNA125a, miRNA125b) that were associated with inflammation were chosen to investigate their roles in CRSwNP.

Isolation and Relative Quantification of miRNAs

Macroarray blocks which contained only nasal polyp areas were used for miRNA extraction. Adjacent normal nasal mucosa was used for miRNA extraction to compare miRNA levels. Total RNA isolation and relative quantification was performed using the method described by Livak et al. (17).

Statistical Analysis

Results were presented as mean ± standard deviation for continuous variables, and defined as percentages for categorical variables. The $\log_2$ transformed fold change data in miRNA-31, miRNA-125a and miRNA-125b expression levels between nasal polyp and normal tissue were analyzed statistically. Statistically significant fold changes in miRNA expression were tested by the inclusion of null value in 95% confidence interval. E-picos calculator (www.e-picos.com) was used for statistical analysis.

Results

A total of 40 patients were studied among 110 patients. The mean age of the patients was 42.1 years. Thirty one of the patients were male and 9 were female. The mean miRNA expression levels of nasal polyp tissues normalized to normal tissues are presented in Table 2. The mean miRNA

Table 1. Baseline characteristics of the patients

Patients, n	40
Mean age (range), years	42.1 (20-87)
Females/males, n	9/31
History of allergic rhinitis	8
History of allergic asthma	1
Previous sinus surgeries, yes/no	15/25

levels of miRNA-31, miRNA-125a, and miRNA-125b were found to be increased in nasal polyp tissues compared to normal nasal mucosa. The mean miRNA levels of miRNA-31, miRNA-125a, and miRNA-125b were 1,698 fold, 2,797 fold, and 1,423 fold, respectively. Using transformed data, the presence of a statistically significant difference in miRNA expression levels between nasal polyp and normal tissue was tested with confidence interval detection (Table 3). There was no statistically significant difference in the expression levels of miRNA-31, miRNA-125a and miRNA-125b in nasal polyp tissue compared to normal tissue. Bootstrap analysis was performed to eliminate an error due to an insufficient sample size for miRNA-125a data that did not conform to the normal distribution (Table 4). There were no different results after Bootstrap analysis.

Table 2. Comparison of the mean micro RNA (miRNA) expression levels in nasal polyp tissue

	95% confidence interval
	Mean miRNA expression change (fold)
miRNA-31	1.698
miRNA-125a	2.797
miRNA-125b	1.423

Table 3. Fold change (log_2 transformed) miRNA-31, miRNA-125a, miRNA-125b expression levels

		95% confidence interval	
	Mean	Minimum	Maximum
miRNA-31	-0.2215	-0.7752	0.3321
miRNA-125a	0.5263	-0.1602	1.2127
miRNA-125b	-0.3183	-0.9142	0.2777

Table 4. Fold change (log_2 transformed) miRNA-31, miRNA-125a, miRNA-125b expression levels after Bootsrap analysis using Statkey

		95% confidence interval	
	Mean	Minimum	Maximum
miRNA-31	-0.2197	-0.7488	0.3135
miRNA-125a	0.5232	-0.1623	1.1409
miRNA-125b	-0.3183	-0.9025	0.2411

Discussion

Chronic inflammation such as allergic rhinitis and CRS cause tissue remodeling in upper airways. CRSwNP progresses with benign edematous polyp formation and sinonasal mucosal thickening. Eosinophil infiltration, chronic mononuclear cells, and epithelial goblet cell hyperplasia are seen in histopathological examinations. An increase in basement membrane, in α-smooth muscle actin (α-SMA), myofibroblasts and submucosal collagen deposition is associated with upper airway remodeling (11,18,19). The etiology of CRS remains to be unknown and CRS etiopathogenesis and its inflammation mechanism is still not well defined. miRNAs are small, 22-nucleotide RNAs. miRNAs control different variety of targeted genes which affect translation and stability of mRNA. Recent researches have shown that miRNAs play very important roles in a wide variety of biological processes for instance in proliferation, differentiation, apoptosis, signal transduction and organ development (12). There are studies aiming to investigate the roles of miRNAs in CRS etiology. Zhang et al. (5) researched the expression levels of miRNA machinery proteins in CRSwNP, CRSsNP and control groups. They found that protein activator of the interferon-induced protein kinase (PACT) mRNA expression was found to be impaired in CRS and PACT was upregulated in CRSwNP as compared to the controls and CRSsNP. Zhang et al. (16) evaluated the expression levels of miRNA-125b in CRSwNP and CRSsNP and reported miRNA-125b was upregulated in CRSwNP. There was no statistically significant difference in the expression levels of miRNA-125b in nasal polyp tissue compared to normal tissue in our study. There may be several reasons for the discrepancy between the two studies. First, Zhang et al. (16) divided CRSwNP cases into eosinophilic and non-eosinophilic cases and found miRNA-125b expression levels higher in eosinophilic CRSwNP cases. We did not divide the CRSwNP cases into two, and this may be one of the reasons for the discrepancy between the two studies. In our study, we compared miRNA levels with nasal polyp tissue and adjacent healthy nasal mucosa in CRSwNP patients. Zhang et al. (16) compared nasal polyp tissue of CRSwP cases and inferior turbinate mucosa of control patients who underwent septoplasty operation. We thought it would be more accurate to compare the miRNA levels in nasal polyp tissue with the adjacent healthy tissue of the same patient. Xia et al. (20) compared expression levels of miRNA-125b and other six miRNAs (miRNA-181b, miRNA-26b, miRNA-155, miRNA-146a, miRNA-, miR-124 and miRNA-92a) in nasal polyp tissue in CRS patients and in nasal mucosa of control patients. They reported miRNA-125b, miRNA-155 and miRNA-146a were up-regulated while miRNA-92a, miRNA-26b and miRNA-181b were down-regulated. There was no study investigating miRNA-31 levels in nasal polyp tissue so far in our literature review. Xia et al. (20) reported miRNA 124 expression levels were not found to have significant changes while Liu et al. (21) reported significantly decreased expression levels of miRNA 124 in nasal polyp tissue. Yu et al. (22) investigated miRNA-663 expression levels in nasal polyp tissue in pediatric population and reported miR-663

expression was significantly decreased in nasal polyp tissue. Luo et al. (23) reported increased expression levels of miRNA-19a in nasal polyp tissue. Li X et al. (11) reported significantly increased levels of miRNA-21 in CRSwNP than those in CRSsNP and controls. There may be several reasons for the discrepancy between our study and the literature. The low number of samples may be one of these reasons. There are no accepted normal ranges for miRNA levels yet. Therefore, in our study, we used the histopathologically normal healthy nasal mucosa adjacent to the nasal polyp tissue of the same patient in order to compare miRNA levels. In other studies in the literature, healthy nasal mucosa of patients without NPs was used to compare the levels of miRNA in nasal polyp tissue of patients with chronic sinusitis. Researchers who designed a study in this way may have considered the nasal mucosa as a single unit and thought that there was no difference between the polyp developing part of the nasal mucosa and the non-developing part in terms of many parameters including miRNA levels. However, in our opinion, the most important weakness of the studies conducted in this way is that there is no accepted normalized value for a certain region of the human body for each miRNAs. Therefore, we think that comparing miRNA levels obtained from nasal polyp tissue of CRSwNP patients with miRNa levels obtained from normal nasal mucosa of healthy individuals is not very reliable.

Conclusion

We could not find any results indicating that miRNA31, miRNA-125a and miRNA-125b would be effective in the pathogenesis of nasal polyp. This result may be due to the fact that we used the same patient's intact nasal mucosa to compare miRNA levels, unlike the literature. A consensus is needed on the comparison of miRNA levels. Studies with larger patient numbers based on this consensus will clarify the role of miRNA levels in nasal polyp pathogenesis.

Ethics

Ethics Committee Approval: Ethics Committee Approval of the study was obtained from of GATA Haydarpaşa Training and Research Hospital (number: 1491-128-13/1539 date: 20/01/2014).

Informed Consent: Written informed consent was obtained from the patients.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Ç., E.E., E.ÇEK., A.G., Concept: E.Ç., E.E., E.ÇEK., İ.Y., G.İ., A.G., Design: E.Ç., E.E., E.ÇEK., İ.Y., G.İ., A.G., E.T., Data Collection or Processing: E.Ç., İ.Y., G.İ., E.T., Analysis or Interpretation: E.T., İ.Y., E.T., Literature Search: E.Ç., E.T., Writing: E.Ç., E.E., E.T.

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Confronting a Pandemic in Early Stages: A Retrospective Analysis From a Pandemic Hospital

Pandeminin Erken Dönemiyle Yüzleşme: Bir Pandemi Hastanesinden Geriye Dönük Analiz

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ABSTRACT

Background: Coronavirus disease-2019 (COVID-19) was spread worldwide by severe acute respiratory syndrome coronavirus-2. We aimed to examine demographic and clinical findings and prognosis of the patients during the first forty days of the pandemic in our country (March 13-April 23, 2020).

Materials and Methods: We analyzed the data of 561 COVID-19 patients hospitalized in a training and research hospital with a 1.607 bed capacity and 253 intensive care beds. Clinical, laboratory characteristics and radiographic findings were recorded and compared between intensive care unit (ICU) and non-ICU groups, and death and survived groups. Binary logistic regression analysis was used to identify independent risk factors for ICU admission and mortality.

Results: The patients' mean age was 53.5±20.3 years, and the median age was 54 years (IQRs: 38-70). 53.7% (n=301) of the patients were male. The average time between the onset of symptoms and admission to the hospital was 3.88 (standard deviation ±3.1) days. The median hospital stay of the patients was eight days (IQRs: 5-11). The most common symptoms in patients were fever [257 (45.8%)], cough [333 (59.4%)], shortness of breath [220 (39.2%)], weakness [148 (26.4%)], and myalgia [130 (23.2%)]. While 21% of the patients (n=118) had at least one comorbid disease, 21.7% (n=122) had more than one additional disease. The most common comorbidities were hypertension, diabetes mellitus and chronic obstructive pulmonary disease, with the rates of 20%, 16.8%, and 15.3%, respectively.

Conclusion: Significant risk factors for ICU care and mortality were as follows: 1. Advanced age, 2. Having coronary artery disease and malignancy, 3. Leukocyte count over ten thousand, 4. Presence of lymphopenia, 5. Elevation of urea and creatinine, C-reactive protein, procalcitonin, Lactate dehydrogenase, D-dimer and cTnI. In our study, the thorax computed tomography played a vital corrective role in patients whose first real-time reverse transcription-polymerase chain reaction test was negative. Also, CURB-65 and qSOFA scores were significantly different in terms of mortality.

Keywords: COVID-19, clinical features, risk factors, prognosis

ÖZ

Amaç: Şiddetli akut solunum sendromu koronavirüs-2 neden olduğu koronavirüs hastalığı-2019 (COVID-19), dünya çapında hızla yayıldı. Bu çalışmada, ülkemizde pandeminin ilk kırk günündeki (13 Mart-23 Nisan 2020) hastaların demografik ve klinik özelliklerinin ve prognozlarının incelenmesi amaçlandı.

Gereç ve Yöntemler: Çalışmamızda, 253 yoğun bakım yatağı olan 1,607 yatak kapasiteli bir eğitim ve araştırma hastanesinde, COVID-19 tanılı 561 hastanın verileri analiz edildi. Yoğun bakım ünitesinde (YBÜ) ve yoğun bakım dışı takip edilen gruplar ile ölen ve hayatta kalan gruplar arasındaki klinik, laboratuvar özellikler ve radyografik bulgular karşılaştırıldı. İkili lojistik regresyon analizi, yoğun bakım ünitesinde takip ve mortalite için bağımsız risk faktörlerini tanımlamak amacıyla kullanıldı.

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ÖZ

Bulgular: Hastaların ortalama yaşı 53,5±20,3 ve ortanca yaş 54 idi (IQRs: 38-70). Hastaların %53,7'si (n=301) erkekti. Semptomların başlaması ile hastaneye başvuru arasında geçen ortalama süre 3,88 (standart sapma: ±3,1) gündü. Hastaların ortanca hastanede kalış süresi sekiz gündü (IQRs: 5-11). Hastalarda en sık görülen semptomlar, ateş [257 (%45,8)], öksürük [333 (%59,4)], nefes darlığı [220 (%39,2)], halsizlik (148 [%26,4)] ve miyaljiydi [130 (%23,2)]. Hastaların %21'i (n=118) en az bir komorbid hastalığa sahipken %21,7'sinde (n=122) birden fazla ek hastalık vardı. En sık görülen komorbiditeler hipertansiyon, diabetes mellitus ve kronik obstrüktif akciğer hastalığıydı (sırasıyla %20, %16,8, %15,3).

Sonuç: Yoğun bakım ünitesinde takip ve mortalite için önemli risk faktörleri şöyle idi: 1. İleri yaş, 2. Koroner arter hastalığı ve maligniteye sahip olmak, 3. On bin üzeri lökosit sayısı, 4. Lenfopeni varlığı, 5. Üre ve kreatinin, C-reaktif protein, prokalsitonin, laktat dehidrogenaz, D-dimer ve cTnI yüksekliği. Ayrıca çalışmamızda toraks bilgisayarlı tomografi, ilk gerçek zamanlı ters transkripsiyon polimeraz zincir reaksiyonu testi negatif olan hastalarda önemli düzeyde tanısal düzeltici rol oynadı. Ayrıca, CURB-65 ve qSOFA skorları mortalite açısından önemli ölçüde farklılık gösterdi.

Anahtar Kelimeler: COVID-19, klinik özellikler, risk faktörleri, prognoz

Introduction

Coronavirus disease-2019 (COVID-19), which started in December 2019 in Wuhan, China, and was spread worldwide by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), caused nearly 13 million confirmed cases and over 550 thousand deaths worldwide by July 2020 (1). More than 210 thousand people have been infected in Turkey, and more than 5300 deaths occurred (2).

Mortality rate was reported as 15% in the first periods. However, as the number of cases increased, this rate varied between 4.3% and 11%. According to the latest data, mortality has decreased to 3.4%. SARS-CoV-2 is more contagious than SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), but the case fatality rate is lower (3). However, the SARS-CoV-2 case death rate is rapidly increasing. Besides, the actual mortality case-death rate is thought to be smaller than it was calculated only in (COVID-19) patients with symptoms severe enough to cause immediate evaluation and hospitalization (4).

Initial reports from affected patient populations in hospitals in China have shown that most patients with severe disease and poor prognosis are accompanied by comorbid conditions such as hypertension, diabetes, obesity, asthma, chronic obstructive pulmonary disease, or advanced age (5,6).

This study was carried out in Kayseri City Hospital located in central Anatolia, a tertiary referral hospital with a total capacity of 1607 beds. The government previously determined the city hospital to provide healthcare for patients during COVID-19 pandemics. Our study aimed to examine demographic and clinical findings and prognosis of the patients during the first forty days of the pandemic.

Material and Methods

In this study, the cases diagnosed with COVID-19 were proven by the clinic and laboratory findings and they were followed-up and treated between March 13 and April 23, 2020, in Kayseri City Hospital, Training and Research Hospital with 1.607 bed capacity and 253 intensive care beds.

Approval was obtained from the Ethics Committee of Kayseri City Hospital for this study (approval no. 76397871/149, approval date: 09.07.2020).

Signed informed consent was exempted due to the retrospective nature of the study.

Inclusion criteria in the study: (1) Patients with a positive real-time reverse transcription-polymerase chain reaction (rRT-PCR) test using throat and nose swab samples with a pre-diagnosis of COVID-19, (2) rRT-PCR test negative during hospitalization, but thorax computed tomography (CT) imaging compatible with coronavirus pneumonia and patients with positive control rRT-PCR test.

Thorax CT classification; The North American Association of Radiology COVID-19 tomography findings are based on the ranking. This classification has a typical, indeterminate or atypical appearance and negative definitions of COVID-19 pneumonia (7). Accordingly, patients with a typical appearance on thorax CT were accepted as coronavirus pneumonia.

Statistical Analysis

In the statistical evaluation of the study's data, categorical data were evaluated as frequency and percentage. Continuous data were assessed as mean ± standard deviation (SD) or median value (minimum-maximum) depending on the data's distribution. The Shapiro-Wilk test was used for normality controls of continuous measurements. After the normal distribution test of continuous variables, two independent group t-tests (independent-sample t-test) were used for the two-group comparisons. For variables that were not compatible with the normal distribution, the non-parametric Mann-Whitney U test was used in two-group comparisons. Binary logistic regression analysis was used to identify independent risk factors for intensive care unit (ICU) and

mortality admission. The significance level (p-value) was taken as 0.05.

Results

Between March 13, 2020, and April 23, 2020, a total of 1639 patients applied to the pandemic unit and emergency clinic pandemic unit of our hospital. One thousand two hundred thirty-one of the patients were hospitalized with the pre-diagnosis of COVID-19. In 235 of the hospitalized patients, the rRT-PCR test taken before hospitalization was positive; whereas 331 had the first rRT-PCR test negative (repeated rRT-PCR tests were positive during hospitalization). Still, there was a typical thorax CT image for COVID-19. A total of 561 patients with positive rRT-PCR test and thorax CT imaging were included in the study.

The patients' mean age was 53.5±20.3 years, and the median age was 54 years inspections' and Internal Quality Reviews (IQRs: 38-70). 53.7% (n=301) of the patients were male. 429 (76.5%) of the patients were hospitalized in the ICU, while 132 (23.5%) were hospitalized in the isolation wards. The average time between the onset of symptoms and admission to the hospital was 3.88 (SD: ±3.1) days. The median hospital stay of the patients was eight days (IQRs: 5-11).

The most common symptoms were fever, cough, shortness of breath, myalgia, weakness, headache, and nausea. The most common symptoms in patients were fever [257 (45.8%)], cough [333 (59.4%)], shortness of breath [220 (39.2%)], weakness [148 (26.4%)], and myalgia [130 (23.2%)]. Less common symptoms were headache [7 (1.2%)], nausea, and vomiting [13 (2.3%)]. A total of 48 patients (8.6%) did not have any symptoms at the time of presentation (Table 1).

While 21% of the patients (n=118) had at least one comorbid disease, 21.7% (n=122) had more than one additional disease. The most common comorbidities were hypertension (HT), 16.8% (n=94) diabetes mellitus (DM) and 15.3% (n=86) chronic obstructive pulmonary disease (COPD) with 20% (n=112). Of the patients included in the study, a total of 9.3% (n=52) deaths occurred. Initial clinical and laboratory characteristics of the patients are shown in Tables 1 and 2.

In our study, antibiotics was administered in 86.5% of the patients, hydroxychloroquine in 30.8%, oseltamivir in 44.9%, and favipiravir in 10.7%. Besides, convalescent plasma (CP) was applied to 1.8% of the patients and stem cell therapy to 3.2%. No medical treatment was given to 7.7% of the patients (Table 1).

Compared patients admitted to the ICU with those admitted to isolation ward, patients admitted to the ICU were significantly older (69.7±14.6 years vs 48.5±19.1 years). In addition, the patients who were admitted to ICU were more likely to have underlying comorbidities including HT (30.3%

Table 1. Clinical and laboratory characteristics of patients with COVID-19

Age, mean (± SD) y	**53.5±(20.3)**	
Gender	**n**	**(%)**
Female	260	(46.3)
Male	301	(53.7)
Isolation ward	429	(76.5)
ICU	132	(23.5)
Died	52	(9.3)
Survive	509	(90.7)
Onset of symptoms to hospital admission, mean ± SD (min-max) day	3.88±3.1 (1-14)	
Length of stay in hospital. median IQRs. day	8 (IQRs: 5- 11)	
Initial signs and symptoms	**n**	**(%)**
Fever	257	(45.8)
Cough	333	(59.4)
Dyspnea	220	(39.3)
Fatigue	148	(26.4)
Myalgia	130	(23.2)
Headache	7	(1.2)
Nausea	13	(2.3)
Asymptomatic	48	(8.6)
Initial laboratory findings	**Median**	**IQRs**
White blood cell count. ×103/uL	7.27	(5.46-10)
Neutrophil count ×103/uL	4.8	(3.39-7.55)
Lymphocyte count ×103/uL	1.55	(1.08-2.1)
Blood urea nitrogen mg/dL	13	(10-20)
Creatinine mg/dL	0.83	(0.66-1.07)
C-reactive protein mg/L	19.8	(5.1-63.9)
Procalcitonin µg/L	0.08	(0.05-0.17)
Lactate dehydrogenase U/L	244	(194-327)
Gamma glutamyltransferase U/L	24	(16-42)
D-dimer mg/L	540	(280-1.207)
Creatine kinase U/L	77	(134.5)
	Mean	**± SD**
Albumin g/L	39.6	5.9
Platelet count ×103/uL	233.3	75.1
Treatment	**n**	**(%)**
Antiviral therapy	-	-
Hydroxychloroquine	173	(30.8)
Favipiravir	60	(10.7)
Oseltamivir	252	(44.9)
Lopinavir-ritonavir	4	(0.7)
Antibiotic treatment	485	(86.5)
Plasma	10	(1.8)
Stem cell therapy	18	(3.2)
Without any treatment	43	(7.7)

IQRs: Inspections' and internal quality reviews, COVID-19: Coronavirus disease-2019, ICU: Intensive care unit, SD: Standard deviation

vs 16.8%), DM (26.5% vs 13.8%), COPD (22.7% vs 13.1%), cardiovascular disease (CVD) (23.5% vs 10.5%), chronic kidney disease (CKD) (4.5% vs 1.2%), and cancer (9.8% vs 0.9%). (p-values= <0.005).

Over the age of sixty-five years, coronary artery disease, malignancy, lymphopenia, and elevation of some laboratory values were determined as significant risk factors related to ICU hospitalization and mortality (Table 3).

There was a statistically significant difference in ICU and mortality between the CURB-65 score, thorax CT groups (typical, indeterminate, atypical, and negative), and diagnosed unit (emergency room vs pandemic) groups. There was no statistically significant difference in ICU and mortality between the onset of symptoms and hospital admission. While there was no statistically significant difference between the qSOFA criteria groups in terms of ICU, there was a statistically significant difference in terms of mortality (Table 2, 4).

There were statistically significant differences in the number of laboratory values such as white blood cell and neutrophil count and D-dimer, procalcitonin, BUN, and creatinine levels in ICU hospitalization and mortality (Table 2, 4).

Discussion

Our study covered 40 days after the first COVID-19 case in our region on March 13, 2020. As shown in Figure 1, there was an increasing trend of hospitalized patients over time. During this first 40 days, it was observed that hospitalization reached its maximum on April 6, 2020. This finding may confirm the rapid spread of the disease in the population. Several studies have reported that the transmission rate (R0) of SARS-CoV-2 infection is between 0.3 and 3.77. This difference can be thought to be due to different sample sizes and possible viral variation (8). Also, super emitters have been reported during SARS and MERS outbreaks. It has been emphasized that focusing on asymptomatic transmitters is required, especially in preventing SARS-CoV-2 spread (8,9). In our study, the rate of the asymptomatic patient group was 8.6%.

Studies conducted in China when the disease was first detected showed that most patients with severe disease and poor prognosis were accompanied by comorbid conditions such as HT, DM, obesity, asthma, COPD, or advanced age (5,6). The majority of 52 deaths in our study were 70 years old and older (n=35), and 34.6% of the patients died with at least one comorbidity such as DM, HT, and COPD.

Mortality rates were reported as 15% in the first periods, but as the number of cases increased, the case fatality rates ranged from 4.3% to 11%. According to the latest data, it has decreased to 3.4% (4). In our study, the case fatality rate was determined as 4.2%.

Older age is a significant risk factor for the death or ICU need of patients with COVID-19 (10,11,12). Also, advanced age and increased comorbidity are independent predictors for COVID-19 patients in in-hospital mortality (13). In our study, common risk factors for ICU care and death were advanced age, coronary artery disease, and malignancy.

Low lymphocyte levels in the disease's diagnosis and throughout the disease were associated with mortality. Also, high D-dimer, troponin I, and LDH values were defined as poor prognostic factors associated with death and severe illness (7,11). In our study, significant risk factors were; 1. leukocyte count above ten thousand, 2. the presence of lymphopenia, 3. high levels of urea and creatinine, CRP, procalcitonin, LDH, d-dimer, and cTnI.

The standard diagnostic method for COVID-19 is the rRT-PCR test with high specificity but low sensitivity. It has been shown that nasopharyngeal swab has a sensitivity of approximately 60%, tracheal aspirates roughly 70%, and bronchoalveolar lavage in the range of 90% to 95% to detect SARS-CoV-2 (14,15). The performance of tests that detect viral RNA depends on the viral RNA present in the sample taken. In practice, negative test results obtained especially with nasopharyngeal and oropharyngeal swabs do not rule out the possibility of COVID-19 infection. This may be due to insufficient viral RNA in the sample due to the low quality of the sample obtained with false-negative PCR results, the collection time of the clinical sample, and the sample's transfer under unacceptable conditions (16).

Another problem is that the rRT-PCR test result takes about two days. Besides, in cases where the rRT-PCR test is challenging to provide, some clinicians may refer to the patient's thoracic CT for the decision because thorax CT can correct the false negativity of rRT-PCR test in the early stage of the disease. However, it should be kept in mind that CT findings may also be normal within the first 2-4 days when symptoms develop (17). In a study, radiologists showed that thoracic CT had high specificity but moderate sensitivity in distinguishing COVID-19 from viral pneumonia (18). In another study, while the initial rRT-PCR sensitivity was only 83%, it was shown that the CT sensitivity was 97% (19). In our research, while the first rRT-PCR test was negative in 59% of the cases, thorax CT was positive, and repeated rRT-PCR tests were positive in their follow-up. The high sensitivity of thoracic CT can explain this situation in the early period and the inexperience had during the first 40 days of taking and testing nasopharyngeal swabs in our study patients. However, in 11% of the patients, while the first rRT-PCR test was positive, thorax CT was negative/normal.

The consisting of confusion, urea level, respiratory rate, blood pressure, and age >65 years (CURB-65) score, which is used to determine the need for hospitalization in adults

Table 2. Basic characteristics of patients with COVID-19

	no (%)			
	Total	**ICU**	**non-ICU**	**p**
Age, mean (± SD). y	53.5±20.3	69.7±14.6	48.5±19.1	<0.001
Age, range y				
<39	157 (28)	5 (3.8)	152 (35.4)	<0.001
40-49	78 (13.9)	4 (3)	74 (17.2)	
50-59	96 (17.1)	19 (14.4)	77 (17.9)	
60-69	87 (15.5)	35 (26.5)	52 (12.1)	
≥70	143 (25.5)	69 (52.3)	74 (17.2)	
Gender				
Female	260 (46.3)	60 (45.5)	200 (46.6)	0.814
Male	301 (53.7)	72 (54.5)	229 (53.4)	
Comorbidities				
Hypertension	112 (20)	40 (30.3)	72 (16.8)	0.001
Diabetes	94 (16.8)	35 (26.5)	59 (13.8)	0.001
COPD	86 (15.3)	30 (22.7)	56 (13.1)	0.007
Cardiovascular disease	76 (13.5)	31 (23.5)	45 (10.5)	<0.001
Chronic kidney disease	11 (2)	6 (4.5)	5 (1.2)	0.014
Malignancy	17 (3)	13 (9.8)	4 (0.9)	<0.001
Number of comorbidities				
0	321 (57.2)	39 (29.5)	282 (65.7)	<0.001
1	118 (21)	44 (33.3)	74 (17.2)	
2 or more	122 (21.7)	49 (37.1)	73 (17)	
CT findings				
Negative/normal	63 (11.2)	4 (3)	59 (13.8)	0.001
Typical	428 (76.3)	104 (78.8)	324 (75.5)	
Indeterminate	49 (8.7)	18 (13.6)	31 (7.2)	
Atypical	21 (3.7)	6 (4.5)	15 (3.5)	
qSOFA criteria				
<2	237 (42.2)	57 (43.2)	180 (42)	0.803
≥2	324 (57.8)	75 (56.8)	249 (58)	
CURB-65 score				
0 or 1	15 (2.7)	2 (1.5)	13 (3)	<0.001
2	498 (88.8)	96 (72.7)	402 (93.7)	
≥3	48 (8.6)	34 (25.8)	14 (3.3)	
Diagnosis unit				
Emergency service	254 (45.3)	94 (71.2)	160 (37.3)	<0.001
Pandemic clinic	307 (54.7)	38 (23.5)	269 (76.5)	
Onset of symptom to hospital admission mean (± SD)	3.88±3.1	3.55±3.01	3.98±3.1	0.171
	Median (IQR)			
White blood cell count ×10^3/uL	7.27 (5.46-10)	9.3 (6.7-14.2)	6.9 (5.3-9.2)	<0.001
Neutrophil count ×10^3/uL	4.8 (3.39-7.55)	7.2 (4.5-11.3)	4.2 (3-6.3)	<0.001
Lymphocyte count ×10^3/uL	1.55 (1.08-2.1)	1.09 (0.76-1.7)	1.66 (1.2-2.2)	<0.001
Blood urea nitrogen mg/dL	13 (10-20)	20 (14-35.5)	12 (9-17)	<0.001
Creatinine mg/dL	0.83 (0.66-1.07)	1.04 (0.76-1.58)	0.8 (0.65-0.96)	<0.001
C-reactive protein mg/L	19.8 (5.1-63.9)	62.5 (33.6-117)	11.6 (3.7-40.9)	<0.001
Procalcitonin µg/L	0.08 (0.05-0.17)	0.20 (0.09-0.65)	0.06 (0.04-0.10)	<0.001
Lactate dehydrogenase U/L	244 (194-327)	297 (228-404)	229 (188-292)	<0.001
Gamma Glutamyltransferase U/L	24 (16-42)	27 (18-51)	21 (15-40.5)	0.017
D-dimer mg/L	540 (280-1.207)	1.250 (672-3.322)	410 (250-805)	<0.001
Creatine kinase U/L	77 (134.5)	83 (59-159)	72 (51-117)	0.134
	Mean ± SD			
Albumin g/L	39.6±5.9	34.9±5.7	41.3±5.05	<0.001
Platelet count ×10^3/uL	233.3±75.1	222.5±80.7	236±73	0.058

IQRs: Inspections' and internal quality reviews, COVID-19: Coronavirus disease-2019, ICU: Intensive care unit, SD: Standard deviation, COPD: Chronic obstructive pulmonary disease, CT: Computed tomography, IQR: Interquartile range

Table 3. Risk factors for ICU care and mortality in COVID-19 patients

	ICU		Mortality	
	OR (95% CI)	**p**	**OR (95% CI)**	**p**
Age years	0.934 (0.921-0.948)	**<0.001**	1.08 (1.06-1.11)	**<0.001**
Age >65 y vs <65 y	5.9 (3.8-9)	**<0.001**	9 (4.5-18.1)	**<0.001**
Female vs male	-	0.814	-	0.579
Comorbidities				
Hypertension	1.7 (1-2.7)	**0.035**	-	0.825
Diabetes	-	0.144	-	0.747
COPD	-	0.161	-	0.303
CVD	1.9 (1.1-3.4)	**0.014**	2.9 (1.4-6)	**0.004**
CKD	-	0.133	-	0.241
Malignancy	9.2 (2.8-29.6)	**<0.001**	19.6 (6.6-58)	**<0.001**
Laboratory findings				
WBC				
≤4 (ref)	-	-	-	-
4-10	-	0.222	-	0.442
>10	5.3 (2-13.8)	**<0.001**	11.3 (1.5-86.1)	**0.019**
Lymphocyte count				
≥1.1 (ref)	-	-	-	-
<1.1	4.9 (3.1-7.6)	**<0.001**	3.8 (2.1-6.9)	**<0.001**
Bun				
≤19 (ref)	-	-	-	-
>19	5.2 (3.2-8.5)	**<0.001**	11.1 (5.4-22.6)	**<0.001**
Creatinine				
≤1.2 (ref)	-	-	-	-
>1.2	4.2 (2.7-6.7)	**<0.001**	6 (3.3-10.9)	**<0.001**
CRP				
<10 (ref)	-	-	-	-
≥10	11.7 (5.8-23.7)	**<0.001**	6.2 (2.4-15.8)	**<0.001**
Procalcitonin				
<0.05 (ref)	-	-	-	-
≥0.05	12.3 (4.3-34.5)	**<0.001**	1.17 (1.1-1.23)	**<0.001**
LDH				
≤245 (ref)	-	-	-	-
>245	2.7 (1.6-4.3)	**<0.001**	3.2 (1.6-6.4)	**0.001**
D-dimer				
≤500 (ref)	-	-	-	-
>500	6 (3.2-11.4)	**<0.001**	10 (3-33.8)	**<0.001**
Creatine kinase				
≤185 (ref)	-	-	-	-
>185	3.4 (1.3-8.7)	**0.010**	-	0.66
Fibrinogen				
Normal (ref)	-	-	-	-
High	3.4 (1.7-6.5)	**<0.001**	-	0.308
cTnI (Troponin I)				
<0.3 (ref)	-	-		
≥3.3	8.9 (2.8-28.2)	**<0.001**	9.18 (3.3-25.5)	**<0.001**

IQRs: Inspections' and internal quality reviews, COVID-19: Coronavirus disease-2019, ICU: Intensive care unit, SD: Standard deviation, COPD: Chronic obstructive pulmonary disease, CT: Computed tomography, IQR: Interquartile range, CI: Continuous interval, CVD: Cardiovascular disease, CKD: Chronic kidney disease, WBC: White blood cell, CRP: C-reactive protein, LDH: Lactate dehydrogenase

Table 4. Basic characteristics of patients with COVID-19

	Total	Death	Survive	p
Age mean (± SD) y	53.5±20.3	74.9±13.8	51.3±19.5	<0.001
Age range y				
<39	157 (28)	1 (1.9)	156 (30.6)	**<0.001**
40-49	78 (13.9)	1 (1.9)	77 (15.1)	
50-59	96 (17.1)	3 (5.8)	93 (18.3)	
60-69	87 (15.5)	12 (23.1)	75 (14.7)	
≥70	143 (25.5)	35 (67.3)	108 (21.2)	
Gender				
Female	260 (46.3)	26 (50)	234 (46)	0.579
Male	301 (53.7)	26 (50)	275 (54)	
Comorbidities				
Hypertension	112 (20)	12 (23.1)	100 (19.6)	0.556
Diabetes	94 (16.8)	11 (21.2)	83 (16.3)	0.373
COPD	86 (15.3)	13 (25)	73 (14.3)	**0.042**
Cardiovascular disease	76 (13.5)	16 (30.8)	60 (11.8)	**<0.001**
Chronic kidney disease	11 (2)	3 (5.8)	8 (1.6)	**0.038**
Malignancy	17 (3)	11 (21.2)	6 (1.2)	**<0.001**
Number of comorbidities				
0	321 (57.2)	12 (23.1)	309 (60.7)	**<0.001**
1	118 (21)	18 (34.6)	100 (19.6)	
2 or more	122 (21.7)	22 (42.3)	100 (19.6)	
CT findings				
Negative/normal	63 (11.2)	2 (3.8)	61 (12)	**<0.001**
Typical	428 (76.3)	30 (57.7)	398 (78.2)	
Indeterminate	49 (8.7)	13 (25)	36 (7.1)	
Atypical	21 (3.7)	7 (13.5)	14 (2.8)	
qSOFA criteria				
<2	237 (42.2)	10 (19.2)	227 (44.6)	**<0.001**
≥2	324 (57.8)	42 (80.8)	282 (55.4)	
CURB-65 score				
0 or 1	15 (2.7)	0 (0)	15 (2.9)	**<0.001**
2	498 (88.8)	36 (69.2)	462 (90.8)	
≥3	48 (8.6)	16 (30.8)	32 (6.3)	
Diagnosis unit				
Emergency service	254 (45.3)	40 (76.9)	214 (42)	**<0.001**
Pandemic clinic	307 (54.7)	12 (23.1)	295 (58)	
Onset of symptom to hospital admission mean (± SD)	3.88±3.1	2.92±2.8	3.97±3.1	**0.020**
	Median (IQR)			
	Total	**Death**	**Survive**	**p**
White blood cell count ×10³/uL	7.27 (5.46-10)	11.8 (7.9-16.5)	7.1 (5.3-9.5)	**<0.001**
Neutrophil count ×10³/uL	4.8 (3.39-7.55)	9.7 (6.3-14)	4.5 (3.2-6.9)	**<0.001**
Lymphocyte count ×10³/uL	1.55 (1.08-2.1)	1.06 (0.76-1.58)	1.6 (1.1-2.1)	**<0.001**
Blood urea nitrogen mg/dL	13 (10-20)	31.5 (18.2-54)	13 (10-17)	**<0.001**
Creatinine mg/dL	0.83 (0.66-1.07)	1.2 (0.9-2.2)	0.8 (0.65-1.0)	**<0.001**
C-reactive protein mg/L	19.8 (5.1-63.9)	67.3 (29.3-188)	16 (4.8-55.9)	**<0.001**
Procalcitonin µg/L	0.08 (0.05-0.17)	0.26 (0.11-0.77)	0.07 (0.04-0.13)	**<0.001**
Lactate dehydrogenase U/L	244 (194-327)	345 (231-435)	238 (190-313)	**<0.001**
Gamma glutamyltransferase U/L	24 (16-42)	27 (16-60)	22 (15-41)	0.123
D-dimer mg/L	540 (280-1.207)	1910 (1.080-5.820)	490 (260-1.010)	**<0.001**
Creatine kinase U/L	77 (134.5)	136 (61-217)	74 (51-118)	**0.046**
	Mean ± SD			
Albumin g/L	39.6±5.9	33.1±6.7	40.5±5.2	**0.029**
Platelet count ×10³/uL	233.3±75.1	244±91.8	232±73.1	0.086

COVID-19: Coronavirus disease-2019, ICU: Intensive care unit, SD: Standart deviation, COPD: Chronic obstructive pulmonary disease, CT: Computed tomography, IQR: Interquartile range

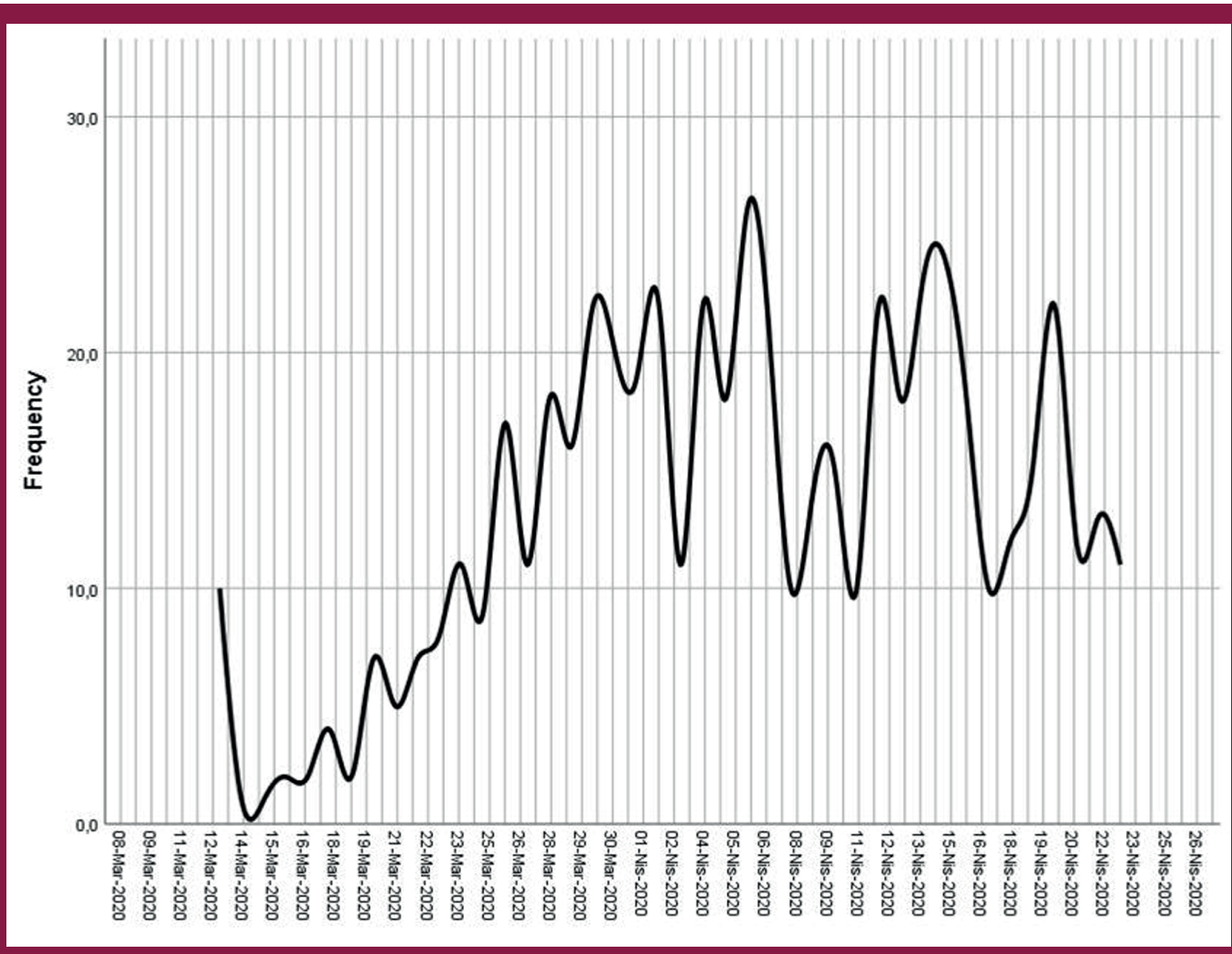

Figure. 1. The distribution of patients according to the dates of hospital admission (n=561)

diagnosed with community-acquired pneumonia, has a low level of evidence (20). Also, the CURB-65 score ranges from 0 to 5. zero-one point shows a low risk for mortality, while 2 points or higher are associated with higher mortality. In a study of 681 COVID-19 patients, it was demonstrated that the CURB-65 score was ≥2, it had a useful, distinctive ability in predicting 30-day mortality, and its sensitivity was 73%, and its specificity was 85% (21). In our study, the CURB-65 score had a significant difference between ICU care and mortality groups.

qSOFA criterion can be used to determine sepsis-induced prognosis in adult patients with suspicious infection in non-hospital, emergency, or general hospital conditions. qSOFA criteria positivity is defined as having at least 2 of the respiratory rate 22 or more minutes, mental state change, or systolic blood pressure 100 mmHg or below (22). A study conducted in 110 hospitalized COVID-19 patients stated that the criteria for systemic inflammatory response syndrome and qSOFA were low in predicting clinical prognosis, and this may be because there are many "silent hypoxemia" patients in COVID-19. Silent hypoxemia describes patients who breathe easily but have low oxygen saturation in pulse oximetry (23). In our study, while there were no differences between ICU care groups in terms of the qSOFA criteria, there were differences between mortality groups.

While the main reason for the rate of increase in COVID-19 cases is the person-to-person transmission, the main reason for the rise in mortality is the lack of a proven medical treatment specific to COVID-19. Medical therapies currently in use are therapies applied to prevent the virus's entry into the cell, inhibit or reduce its replication, and suppress the increased inflammation response. Besides, CP treatment, which includes antibodies of infected and recovered patients, is among the options (24). The uncertainty of treatment during

pandemic and the lack of information on viral pathogenesis challenged the procedure applied in our study.

Hydroxychloroquine and oseltamivir inhibit the entry of the virus into the cell. However, with the recent studies, the evidence showing hydroxychloroquine's *in vitro* activity against SARS-CoV-2 is limited (25). Oseltamivir is a neuraminidase inhibitor used in influenza treatment. Concurrent influenza infection was detected in approximately 4.3% of COVID-19 patients. However, the place of oseltamivir in the treatment of COVID-19 is controversial (26). Due to the high frequency of influenza infection between March and April, our study rate of using oseltamivir was high when our study was conducted.

Due to the limitations experienced in the first period in our region, we could not apply the necessary amounts of favipiravir and remdesivir treatments among treatments that inhibit or reduce the virus's replication. The same reason was valid for the procedures applied to suppress the increased inflammation response.

The World Health Organization and the American Food and Drug Administration stated that the use of CP containing anti-SARS COV-2 antibody could be effective against infection. A guideline has been prepared for the preparation and clinical use of CP in our country. CP usage criteria include the presence of pneumonia with diffuse bilateral involvement in thorax CT, the need for mechanical ventilation, and having poor prognostic parameters (27). CP was applied to ten patients in our study. Mesenchymal stem cells contain multipotent stromal cells that support immunomodulation and regeneration. Stem cells show antiviral activity by suppressing viral replication, viral transmission and viral lung epithelial cell damage. And stem cell therapy is safe and could benefit COVID-19 patients with hypoxic respiratory failure and ARDS (28,29). In our study, stem cell therapy was applied to eighteen patients.

Study Limitations

Our study's significant limitations include its being retrospective, having short working time, and being performed only on hospitalized patients. Secondly, this study was conducted in a single-center tertiary hospital with limited sample size. Therefore, this study is likely to include patients with a disproportionately poor prognosis.

Also, since our study included the 40 days after the first COVID-19 case detected in our region, there were limitations related to patient management, deficiencies in the acquisition or evaluation of rRT-PCR tests and the treatment algorithm.

Conclusions

In our study, the essential risk factors related to critical care and mortality were: 1. Advanced age, 2. Having coronary artery disease and malignancy, 3. Leukocyte count over ten thousand, 4. Presence of lymphopenia, 5. Elevation of urea and creatinine, CRP, procalcitonin, LDH, d-dimer and cTnI. In our study, thorax CT played a vital corrective role in patients whose first rRT-PCR test was negative. As well, the CURB-65 and qSOFA scores were substantially different in terms of mortality.

Highlight Key Points

While the COVID-19 pandemic is still ongoing, our study covers the first 40 days. During this period, both the increase in knowledge about the disease and the patient approach change shown undeniable differences.

Our first focus was to retrospectively address the early stages of the long pandemic process and examine the diagnostic difficulties, patient follow-up, treatment approaches and results of the first period.

Another critical point was the flaws in the diagnostic process. Due to different reasons, lower rRT-PCR sensitivity than thoracic CT sensitivity causes diagnostic delays and errors.

Finally, low lymphocyte levels were associated with mortality in the diagnosis. Throughout the disease, high D-dimer, troponin I and LDH values were defined as poor prognostic factors associated with death and severe illness.

Ethics

Ethics Committee Approval: Approval was obtained from the Ethics Committee of Kayseri City Hospital for this study. (approval no. 76397871/149, approval date: 09.07.2020)

Informed Consent: Signed informed consent was exempted due to the retrospective nature of the study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.K.T., Concept: A.K.T., Design: A.K.T., Data Collection or Processing: A.K.T., E.E., Z.B.D., İ.T., E.S., İ.Ç., Analysis or Interpretation: A.K.T., A.U.K., İ.T., İ.Ç., Literature Search: E.E., Z.B.D., Writing: A.K.T., A.U.K., İ.T., İ.Ç.

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