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CONTENTS

REVIEW

CRISPR-based Approaches for the Point-of-Care Diagnosis of COVID19
İhsan Alp Uzay, Pervin Dinçer 1

ORIGINAL ARTICLES

Investigation of Mean Platelet Volume as a Prognostic Criterion in Non-Healing Wounds
Fatma Nilay Tutak, Fatih Doğan, Aşkı Vural 15

Publication Status of Urology Theses in Turkey
Emrullah Söğütdelen; Mustafa Küçükyangöz 24

Evaluation of Sleep Quality with Use of Angiotensin Receptor Neprilysin Inhibitor in Patients with Reduced Ejection Fraction Heart Failure
Ferhat Işık, Metin Okşul, Burhan Aslan, Ercan Taştan 30

The Potential Use of Elastic Tissue Autofluorescence in Formalin-fixed Paraffin-embedded Skin Biopsies
Deniz Ateş Özdemir, Kader Susesi 37

Comparison of Clinical Profiles, Angiographic Features and Outcomes of Young and Elderly Patients with ST-Segment Elevation Myocardial Infarction
Bektaş Murat, Selda Murat 44

The Relationship Between ST-Segment Depression in Lead aVR and Coronary Microvascular Function in Acute Inferior Myocardial Infarction
Burhan Aslan, Mehmet Zülküf Karahan 53

The Factors Related to the 6 Minute Walk Test: The Experience of a Referral Lung Transplantation Center
Pınar Atagün Güney 59

Distribution of Intestinal Parasites Detected in Ankara Training and Research Hospital between 2017 and 2020
Filiz Kaya, Mustafa Kocaağa 66

e-ISSN: 2147-9488

Comparison of Favipiravir to Hydroxychloroquine Plus Azithromycin in the Treatment of Patients with Non-critical COVID-19: A Single-center, Retrospective, Propensity Score-matched Study

Oğuz Abdullah Uyaroğlu, Meliha Çağla Sönmezer, Gülçin Telli Dizman, Nursel Çalık Başaran, Sevilay Karahan, Ömrüm Uzun.....73

The Effect of Different Therapeutic Modalities on Demodex Densities and Clinical Symptoms of Patients with Demodicosis

Başak Yalıcı Armağan, Nilgün Atakan.....83

CASE REPORTS

Dyspnea and Dysphagia as First Sign of Hypopharyngoesophageal Lipoma

Monika Adásková, Katarína Obtulovičová, Marian Sičák.....90

Anesthetic Management with Dexmedetomidine During the Awake Craniotomy Surgery: A Case Report

Şennur Uzun, Murat İzgi, Turana Rasulova.....95

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REVIEW

CRISPR-based Approaches for the Point-of-Care Diagnosis of COVID19

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ABSTRACT

Severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2), is a novel Betacoronavirus variant that emerged in December 2019 causing the coronavirus disease 19 (COVID19) pandemic. It is reported that asymptomatic and presymptomatic individuals can transmit the virus and this silent transmission has been a major obstacle for the control of the pandemic. To overcome this obstacle, widespread testing with a rapid turnaround time is required. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is currently the golden standard for the diagnosis of COVID19 worldwide. Even though RT-qPCR is an efficient method in terms of sensitivity and specificity, the need for elaborate instrumentation and skilled personnel restricts its widespread use. Restriction of RT-qPCR to a limited number of laboratories makes it further time-consuming. Many approaches are present to address the requirement for a rapid and accurate COVID19 diagnosis. In this review, different CRISPR-based approaches for the point-of-care diagnosis of COVID19 are compared. Among these approaches, CRISPR-FDS on-chip assay is found to be the best option as it is reported to be highly sensitive and specific, has a short turnaround time (15 min), does not need RNA isolation or special tools, and simple to perform. In terms of clinical validation, SHERLOCK, STOPCovid, and DETECTR were the most extensively studied ones and they are also reported to be highly sensitive and specific compared to RT-qPCR.

Keywords: COVID19, SARS-CoV-2, CRISPR-based diagnostics, point-of-care diagnosis, next-generation molecular diagnostics

INTRODUCTION

In December 2019, a group of patients from Wuhan, China were diagnosed with pneumonia of unknown cause [1]. Next-generation sequencing of patient specimens demonstrated that it was a novel Betacoronavirus variant [2], currently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is thought to have resulted from an initial zoonotic transmission event [3] and later spread through person-to-person transmission [4]. Symptoms at the onset of illness are fever, dry cough, myalgia, fatigue, and some patients have mild symptoms or asymptomatic [5,6]. After the viral transmission, individuals undergo a period of incubation. The median incubation period was estimated to be 5,1 days (95% CI, 4.5 to 5.8 days), and 97,5% of the symptomatic patients developed symptoms within 11.5 days (95% CI, 8.2 to 15.6 days) of infection [7]. Patients may show positive reverse transcription-quantitative polymerase chain reaction (RT-qPCR) test results during the incubation period, suggesting that these individuals might be infectious before becoming symptomatic [8]. Presymptomatic transmission is further supported by many case studies [9,10]. A viral dynamics study estimated the proportion of presymptomatic transmission as 44% (95% CI, 30-57%) [11]. It is reported that asymptomatic individuals have similar viral titers with symptomatic

individuals [12] and they can transmit the virus [13,14]. This silent transmission has been a major obstacle for the control of the pandemic since the emergence of the virus, proving the importance of detecting asymptomatic and presymptomatic individuals through widespread testing [15].

COVID19 has a significant mortality and morbidity burden on society and accurate COVID19 diagnosis is one of the key steps to contain the pandemic. There are currently many tests available for the diagnosis of COVID19 such as serological tests, computerized tomography (CT), and RT-qPCR tests. Serological tests, one of the first tests applied for COVID19 diagnosis, aim to detect serum antibodies against SARS-CoV-2 Spike protein. It is an important test in terms of following the immune response resulting from the infection or vaccination. However, it is not well suited for the acute phase diagnosis [16] since IgM/IgG production starts from 4 days after the onset of symptoms [17] and shows a rapid increase at 6-7th days [18]. Another diagnostic approach, CT scan is a highly sensitive alternative to RT-qPCR. It is reported in two meta-analysis that CT scan has the sensitivity of 87% (95% CI, 85-90%) [19] and 91.9% (95% CI 89.8%-93.7%) [20] compared to RT-qPCR. In a study with 1014 patients, it was reported that 60-93% of the cases had initial positive CT result before positive RT-qPCR result [21]. Despite these features, a CT scan has the disadvantage of having low specificity. According to two aforementioned meta-analysis, CT scan has the specificity of 46% (95% CI, 29-63%)[19] and 25.1% (95% CI, 21.0%-29.5%)[20].

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is currently considered the golden standard for the diagnosis of COVID19 [22,23]. However, the test has many shortcomings. First of all, it is stated that RT-qPCR has many pre-analytical and analytical vulnerabilities that can jeopardize its results [24]. Furthermore, false-negative results have been reported [21,25] which raise the suspicion of the RT-PCR test as the golden standard diagnostic tool for COVID19 [26]. Starting from sample preparation to final readout, processing of conventional RT-qPCR requires 4-6 hours [27]. However, the overall turnaround time is much longer. According to a large survey in the United States (with 19,058 responders), the average wait time for the test result was 4,1 days [28]. In Turkey, average wait time for the test result is estimated to be1-2 days (Unpublished data). This experienced delay is thought to have resulted from high-frequency testing and limitation of RT-qPCR to central laboratories. RT-qPCR requires elaborate and expensive instrumentation and technical expertise which increase its cost and limit its widespread use. Therefore, a point-of-care diagnostic test with a rapid turnaround time remains to be an urgent requirement to contain the pandemic.

In this review, we will compare some of the many CRISPR-based approaches developed for the diagnosis of COVID19 in terms of sensitivity, specificity, and turnaround time. We will discuss the workflow of each test and try to give an idea about their applicability as point-of-care diagnostic tools.

Rapid Detection of SARS-CoV-2 Nucleic Acids

Besides the aforementioned diagnostic tests, there are many rapid COVID19 tests suitable for point-of-care diagnostics. These rapid tests include RT-LAMP tests [29], Rapid Antigen Tests [30], and CRISPR-based approaches [31]. Among these approaches, nucleic acid detection methods mainly rely on isothermal amplification methods instead of conventional PCR to free the test from the restriction of having a thermocycler. One of the rapid nucleic acid detection systems is Abbot ID Now™. It is an automated system taking advantage of isothermal amplification and approved by the United States Food and Drug Administration (U.S. FDA) [32] as a point-of-care diagnostic test. However, the overall positive percent agreement of Abbot ID Now with RT-qPCR was found to be 73.9% [33] and 75% [34] in two studies. U.S. FDA recommends confirmation of negative results with a high-sensitivity authorized molecular test [35]. Another U.S. FDA-approved rapid nucleic acid detection system, Cepheid Xpert Xpress™, had 98,9% positive percent agreement with RT-qPCR [33].

RT-qPCR

RT-qPCR is currently the golden standard for the diagnosis of COVID19 and it is the main diagnostic approach worldwide [22,23]. RT-qPCR is a technique, in which amplification and detection processes are combined in a single step with the help of fluorescent chemistry [36]. An RT-qPCR reaction is characterized by the time point when the

amplification of the target is first detected [37]. This time point is called as cycle threshold (Ct). When there is higher nucleic acid initially, amplification is detected at a lower Ct [38]. In RT-qPCR protocol, the viral genome is first extracted from the patient samples. Then, target viral genes are converted to cDNA by reverse transcription (RT) and amplified by polymerase chain reaction (PCR). Amplified products can be detected through various methods such as DNA binding dyes, hydrolysis probes, hybridization probes, etc. DNA binding dyes (SYBR Green I) bind to dsDNA that is formed during the reaction and they are not sequence-specific [39]. It is cheaper than sequence-specific probes but it has some specificity issues [37]. When the dye binds to primer-dimers or non-specific PCR products, it may produce false-positive results [40]. According to the United States Centers for Disease Control and Prevention (U.S. CDC) guideline, primer-probe sets targeting the Nucleocapsid (N) gene of the SARS-CoV-2 are used [41]. Other primer-probe sets recommended by the China CDC, Hong Kong University, and World Health Organization (WHO) have also been proven to have enough analytical sensitivity [42]. RT-qPCR has an analytical limit of detection (LOD) of 1,000 viral copies/mL (1 copy/µL) [42]. There are many different findings in terms of sensitivity and specificity of RT-PCR and results vary according to the chosen patient specimen. Overall, RT-qPCR testing of lower respiratory specimens (Bronchoalveolar lavage fluid and sputum) provide the highest sensitivity [43,44]. It is stated in a meta-analysis that, RT-PCR test has the sensitivity of 97,2% (95% CI, 90,3-99,7%) with sputum, 73,3% (95% CI, 68,1%-78%) with Nasopharyngeal/Oropharyngeal swab and 62% (95% CI, 54,5%-69,9%) with saliva sample [20]. False-negative results with RT-PCR are reported [21,25] and in such cases with clinical suspicion, testing with RT-qPCR for the second time or confirmation with CT scan is recommended [19,27]. Further systematic analysis and better reference standards are needed for the comparison of RT-qPCR.

CRISPR based Diagnostic Tests for COVID19

Clustered regularly interspaced palindromic repeats (CRISPR) were first identified by Japanese researchers in 1987 as "short direct repeats interspaced with short sequences in the genome of Escherichia coli" [45] and later found out to be present in many prokaryotes [46]. CRISPR and CRISPR-associated (Cas) proteins are together responsible for the prokaryotic adaptive immune system against bacteriophages and plasmids [47,48]. This bacterial defense mechanism was repurposed for many purposes including genome editing, transcriptional perturbation, and elucidation of gene function [49,50]. Among its applications, nucleic acid detection is proven to be highly sensitive and specific for the diagnosis of many viral and bacterial diseases [51-53].

There are many different CRISPR-based approaches for the diagnosis of COVID19. Each approach follows a different workflow as depicted in Figure 1. Starting from the specimen collection, different patient samples like saliva, sputum, nasopharyngeal, oropharyngeal, and nasal swabs can be chosen. Then, viral RNA must be extracted, and this extraction can be done through either conventional RNA isolation methods (like spin-column based isolation) or through simpler methods (like magnetic-bead assisted isolation). Extracted viral nucleic acid is later amplified through various isothermal amplification methods and readout is obtained with a lateral flow assay or with a fluorescent reporter. We will now discuss each CRISPR-based diagnostic approach and compare their results. Results obtained from each test can be seen in Table 1.

CRISPR-Cas12 based COVID19 Diagnostic Tests

CRISPR-Cas12 is an RNA-guided DNase that displays non-targeted strand cleavage (collateral cleavage) upon detection of the target sequence [54]. When the designed CRISPR-RNA (crRNA) matches with the targeted DNA sequence, Cas12 cleaves the target strand along with the nearby ssDNA and dsDNA oligonucleotides. [54]. This collateral cleavage activity is non-specific, and it can be detected through the cleavage of a quenched fluorophore ssDNA reporter or with a lateral flow strip.

4.1.1 STOPCovid.v1 and v2

Sherlock Testing in one pot (STOP) for COVID19 diagnosis is a CRISPR-Cas12 based approach for the diagnosis of COVID19 [55,56]. There are two versions of the test, and they differ in their RNA isolation methods. Joung et al. first developed a protocol that can be done in a single fluid handling step and named the approach as STOPCovid.v1 [56]. They demonstrated that nucleic acid amplification

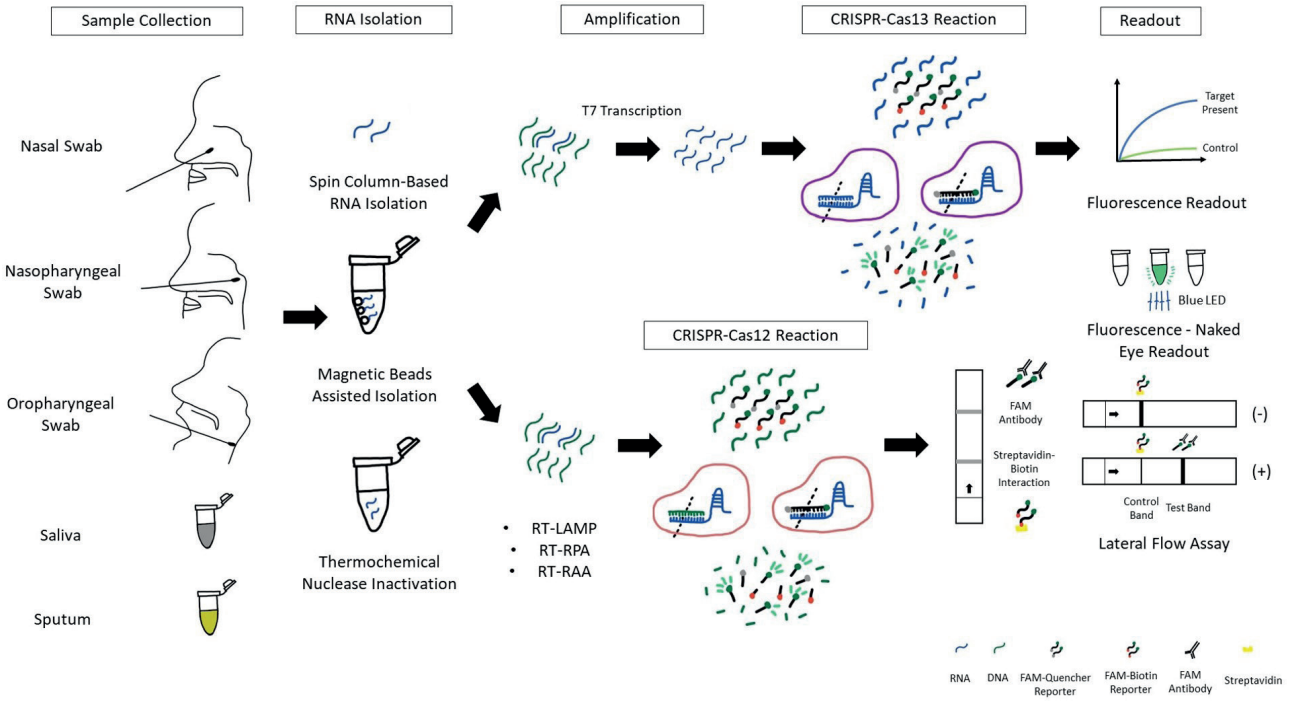

Figure 1. Different approaches are available in each step of the CRISPR-based COVID19 diagnostic tests' workflow. Please refer to the text for further information. For simplicity, Fluorophore-quencher reporter and FAM-Biotin reporter are drawn together in the CRISPR-Cas reaction. Please note that these two reporters represent different readout approaches and are not used together.

Table 1. CRISPR-based diagnostic tests. Sensitivity and specificity are results compared to the RT-qPCR test.

Diagnostic Test	Sample-to Answer Time	Limit of Detection	Tested Patient Samples	Sensitivity	Specificity	Ref.
RT-qPCR	2-8 h	1 copy/µL	-	-	-	[20,27,42]
opvCRISPR	~45 min	5 copies/reaction	50	100%	100%	[66]
DETECTR	30-40 min	10 copies/µL	83	95%	100%	[58]
RCSMS	40 min	5 copies/reaction	276	93,80%	99%	[62]
STOPCovid.v2	15-45 min	0,033 copies/µL	402	93,10%	98,50%	[55]
AIOD-CRISPR	40 min	4,6 copies/µL	-	-	-	[63]
CASdetec	1 h	10 copies/µL	-	-	-	[64]
CRISPR-FDS on Chip Assay	15 min	0,38 copies/µL	103	98,7-100%	100%	[67]
CRISPR-COVID	40 min	2,5-7,5 copies/reaction	114	100%	100%	[74]
SHERLOCK	<1h	10-100 copies/µL	534*	96%	100%	[70][72]
SHINE	50 min	10 copies/µL	50	90%	100%	[73]
Fozouni et al.	<30 min	100 copies/µL	-	-	-	[87]

RNA isolation is not included in these turnaround times except for the ones that are RNA-isolation-free (CRISPR-FDS and RCSMS). Three of the tests (CRISPR-COVID, opvCRISPR, and RCSMS) have different statements for the limit of detection as copies/reaction. *Clinical validation was performed by different research groups.

and CRISPR detection can be performed in a single step. It is a significant result because when we do not need to transfer the amplified product into a second tube for CRISPR detection, contamination risk reduces. The method consists of three steps: Lysis of the virus-containing patient sample using QuickExtract™ to release the viral RNA, detection of viral RNA using STOPCovid master mix (which includes RT-LAMP and Cas12 reagents together). Taurine can also be added to the kit to improve the reaction kinetics. The last step is the visual readout step. A commercially available (Lateral flow dipsticks) paper dipstick can be used (which gives us a result like a pregnancy test). Both NP/OP and saliva samples can be used for the detection of SARS-CoV-2, as it was previously reported that saliva samples have similar viral loads to nasopharyngeal swabs [57].

In STOPCovid.v2, a simpler RNA extraction method with a magnetic bead is performed [55]. This method is thought to be faster and less contamination prone than the conventional RNA extraction methods. Therefore, it is a feasible option for a point-of-care diagnostic test. After the RNA extraction, the RNA genome is reverse transcribed and amplified by LAMP. Isothermal amplification is also a suitable approach for point of care diagnosis because only the presence of a heat block would be enough. LAMP operates at 55-70°C so a thermostable Cas12 enzyme, Alicyclobacillus acidiphilus Cas12b (AapCas12b), is used in the test. The test can reliably detect 33 copies/mL, which is the one-thirtieth of the RT-qPCR method (1000 copies/mL). STOPCovid.v2 has the best limit of detection among the CRISPR-based approaches reviewed in this article. The test has been applied in 402 patient samples in multiple centers. The test has the sensitivity and specificity of 93,1% and 98,5% respectively, compared to the RT-qPCR method.

DETECTR

Broughton et al. developed a method for the detection of COVID19 through CRISPR-Cas12 collateral cleavage activity [58] and shared the protocol with the public in February 2020 [59]. The method is named SARS CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter [DETECTR]. DETECTR received emergency use authorization (EUA) from U.S. FDA on July 9, 2020 [60].

In the DETECTR assay, the extracted viral RNA from the patient sample is amplified and reverse-transcribed into cDNA by RT-LAMP reaction at 62°C for 20-30 minutes. If the targeted SARS-CoV-2 genes are present, Cas12 cleaves the amplified single-stranded DNA together with the nearby FAM-biotin reporter. The test result is obtained through a lateral flow strip by which we can understand if the reporter is cleaved or not. The lateral flow strip provides us a qualitative result such as positive or negative. If the targeted SARS-CoV-2 genes: E and N genes are both detected with Cas12 reaction, the assay is considered as positive. If only one of these genes is detected, the result is considered as presumptive positive. The assay took 30-40 minutes to complete, and the limit of detection was 10 copies/µL. The positive predictive agreement and negative predictive agreement of SARS-CoV-2 DETECTR relative to the CDC RT-qPCR assay were 95% and 100%, respectively, for the detection of SARS-CoV-2 in 83 total respiratory swab samples [58]. This result was further supported by a multicenter study. 378 patient samples were tested with DETECTR and positive predictive agreement of the test relative to RT-qPCR was found to be 95% [61]. DETECTR is a two-tube test, RT-LAMP and Cas12 detection are performed in different tubes. This may be considered as a disadvantage due to contamination risk.

Recently, a locally adapted variant of DETECTR has been developed and named Rapid Coronavirus-Sensitive Monitoring from Saliva (RCSMS) [62]. It is tested in 276 patients. Researchers demonstrated that a low-cost thermochemical treatment with TCEP/EDTA could be sufficient to inactivate nucleases in saliva and eliminate the need for viral RNA extraction. RCSMS was able to detect 5 copies/reaction in 40 minutes and had the sensitivity and specificity of 93.8% and 99.0% respectively, relative to RT-qPCR.

AIOD-CRISPR

All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR) Assay is developed by Ding et al. and the significant difference of their approach from other CRISPR-based tests is that they combined two crRNAs to improve the sensitivity of the test [63]. Although combining crRNA1 and crRNA2 did not result in a different fluorescence signal than crRNA2 alone, dual crRNA was able to detect a lower amount of SARS-CoV-2 genomic material. It is a single tube test [like STOPCovid] that does not require a transfer process. The preferred amplification method was RPA, and the readout can be obtained through both fluorescence signal and direct observation without a device, so-called naked-eye readout. AIOD-CRISPR method has been tested for the detection of both HIV and SARS-CoV-2 viruses. When they tested detection of the SARS-CoV-2 N gene on a plasmid, visual detection was obtained in 40 minutes. There was no cross-reaction (with other coronaviruses) which shows the specificity of the method. A high concentration of ssDNA-FQ reporters strengthened the signal for detection. Researchers did not perform a clinical validation for the method.

CASDetec

CRISPR-assisted detection (CASdetec) is a method employing CRISPR-Cas12b for the diagnosis of

COVID19 [64]. The detection limit of CASdetec is 10.000 copies/mL (10 copies/µL). Researchers have reported that increasing the crRNA concentration increased the rate of reaction and resulted in an enhanced fluorescence signal. They have previously demonstrated that CRISPR was unable to detect target DNA when there was <1-10nM of amplification product [65] and preferred to amplify the genomic material with RT-RAA.

opvCRISPR

One-pot visual reverse transcription (RT)-LAMP-CRISPR (opvCRISPR) test is a sensitive method with a simplified operation that implements CRISPR/Cas12a for the detection of SARS-CoV-2 [66]. RT-LAMP is used for amplification and the readout is obtained with quenched fluorescent single-stranded DNA (ssDNA) reporter. A fluorescent signal is made visible to the naked eye with the help of blue light. The significance of the opvCRISPR method is that it is shown to be superior to colorimetric RT-LAMP techniques for the diagnosis of COVID19 in terms of sensitivity and specificity. To confirm that Cas12a cleavage improves the sensitivity of the test, RT-LAMP amplification was stopped at different time points and products were cleaved with Cas12a. It is demonstrated that within 20 minutes of amplification, amplicons were below the fluorescence threshold and could not be detected with RT-LAMP alone. But Cas12a cleavage provided enough fluorescent signal within 20 minutes, thus increasing the sensitivity of RT-LAMP, and shortening the required time. The test was able to detect SARS-CoV-2 at nearly the single-molecule level and can be accomplished in 45 minutes. The method demonstrated 100% positive predictive agreement with RT-qPCR.

CRISPR-FDS on Chip Assay

CRISPR-FDS is a method that uses Cas12a for the detection of COVID19 in patient saliva specimens [67]. It does not require a separate RNA isolation step. Instead, they optimized the lysis step to make the saliva specimen compatible with CRISPR reagents. That way, the procedure is shortened, and the test is freed from expensive reagents required for RNA isolation. Lysis of the patient sample is followed by RT-RPA amplification and a CRISPR-Cas12a reaction that can be detected with fluorescent reporters. For the interpretation of the fluorescent signal, the group devised an on-chip assay that can be read by a smartphone fluorescence reader.

To summarize the workflow, saliva samples are collected into tubes that contain lysis buffers and heated for 5 minutes. Afterward, lysed samples are added to the sample wells of the assay chip. Assay Chip contains five wells that are preloaded with premixed RT-RPA and CRISPR-Cas12a reagents. The chip is then incubated for 10 minutes at room temperature and inserted into the smartphone reader. A smartphone fluorescence reader is a 3D printed device that contains a laser diode, and the results can be visualized with a smartphone. It is a portable and inexpensive device that is suitable for point-of-care testing. Overall, the sample-to-answer time was 15 minutes which is the shortest duration compared to other nucleic acid detection methods. The limit of detection of the test was 0,38 copies/µL, which occurs to be the second-best (after STOPCovid.v2) among the nucleic acid detection methods reviewed in this article.103 clinical specimens were tested with CRISPR-FDS. Saliva samples exhibited a false negative rate of 1,3% compared to RT-qPCR. However, swab samples showed complete concordance with RT-qPCR.

The group demonstrated significant results in terms of specimen collection. They demonstrated that in early periods of the SARS-CoV-2 infection, saliva retains SARS-CoV-2 for a longer time than the nasopharyngeal sample in non-human primates.

CRISPR-Cas13 based COVID19 Diagnostic Tests

CRISPR-Cas13 is a RNA-guided RNase which displays collateral cleavage activity [52,68,69]. The main difference between Cas13 and Cas12 is that Cas13 detects and cleaves only single-stranded RNA molecules. When the designed CRISPR-RNA (crRNA) matches with the targeted single-stranded RNA sequence, Cas13 cleaves the targeted ssRNA along with the nearby ssRNA oligonucleotides. Cas13 reaction can be detected just like the Cas12 activity, with a quenched fluorophore ssRNA reporter or a lateral flow assay. As Cas12 detects ssRNA oligonucleotides, the gene of interest is first reverse transcribed into cDNA and amplified by LAMP or RPA. The amplicon is then transcribed back to RNA oligonucleotides by T7 transcriptase, so-called in vitro transcription.

SHERLOCK

Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) is a CRISPR-Cas13a based nucleic acid detection method developed by Gootenberg et. al initially for the detection of Zika and Dengue virus [68]. SHERLOCK method provides rapid nucleic acid detection with attomolar sensitivity and single-base mismatch specificity. Shortly after the emergence of COVID19, Zhang et. al published a SHERLOCK Protocol for the detection of SARS-CoV-2 [70]. The protocol consists of isothermal amplification of isolated viral RNA with reverse transcription-recombinase polymerase amplification (RT-RPA) kit, in vitro transcription of the amplified nucleic acid with T7 transcriptase, and detection of the viral RNA sequence with Cas13a. Finally, a naked-eye readout of the test result is obtained by using a paper dipstick. The test was able to detect target sequences with a concentration between 10-100 copies/µL. The test takes less than an hour to perform and does not require elaborate instrumentation. SHERLOCK became the first CRISPR-based approach to receive emergency approval from the U.S. FDA for the diagnosis of COVID19 [71] (May/2020).

In another study, the SHERLOCK protocol was clinically validated with 534 patient samples [72]. It is reported that the test was 100% specific and 96% sensitive with the fluorescence readout and 88% sensitive with lateral flow readout. The limit of detection of the set with the designed crRNAs against the N gene was found to be 42 copies/reaction, which is concordant with the SHERLOCK study. They also devised the lateral flow assay and included an internal RNase to control contamination.

SHINE

SHERLOCK and HUDSON Integration to Navigate Epidemics (SHINE) is a CRISPR-Cas13a based approach for the detection of SARS-CoV-2 [73]. It does not require prior RNA isolation. The preferred RNA extraction method is heat and chemical reduction to inactivate RNases. Clinical validation of SHINE in 50 patient samples showed 90% sensitivity and 100% specificity compared to RT-qPCR.

CRISPR-COVID

CRISPR-COVID is among the first CRISPR-based tests developed for COVID19 diagnosis [74]. It detects the RT-RPA amplified genomic material with Cas13a Endonuclease activity. The targeted SARS-CoV-2 genomic sequence is Orf1ab, which was selected because it is conserved among reported SARS-CoV-2 genomic variants, and it does not cause a false-positive result with other microorganisms. The method is tested in 114 patient samples, of which 52 were SARS-CoV-2 positive (detected with Metagenomic Next Generation Sequencing) and 62 were negative. CRISPR-COVID was able to detect 52/52 of the positive cases and it gave negative results with 62/62 of negative cases. Using metagenomic next-generation sequencing (mNGS) and PCR as the reference, CRISPR-COVID had the sensitivity and specificity of 100%. Compared to CRISPR-COVID, the PCR test was able to detect 47/52 of the SARS-CoV-2 positive patients. Furthermore, the test took CRISPR-COVID 40 minutes (30 minutes amplification and 10 minutes of Cas13a reaction) to give a result. Whereas mNGS takes approximately 20 hours. The limit of detection of the test was reported to be near a single copy/sample. Among 10 replicates, the test was able to detect all of them with the concentration of 7,5 copies/sample and it was able to detect 6/10 samples with a concentration of 2,5 copies/reaction.

Comparison of Different Workflows

As it can be seen from Table 1, each CRISPR-based diagnostic test achieved a different outcome. Different results are present since many different techniques are available in each step of the workflow. Different methods used in each step of CRISPR-based COVID19 tests are summarized in Table 2.

Sample Collection

It has been reported that positive RT-PCR can be obtained from patient bronchoalveolar lavage fluids, nasal swabs, pharyngeal swabs, feces, and blood [75]. Viral titers of patient throat swab and sputum samples peak around 5-6 days after the onset of symptoms, reaching 104-107 copies/ mL [8]. The virus can be detected shortly after the onset of symptoms and higher viral loads were detected in the nose than in the throat samples [12]. The viral load of asymptomatic individuals was similar to that of symptomatic individuals, which suggests the asymptomatic transmission [12].

Table 2. Different workflows of the CRISPR-based diagnostic tests.

Diagnostic Test	Sample	RNA Isolation	CRISPR	Target Gene	Amplification	Detection	Ref.
opvCRISPR	NP	Not Specified	Cas12a	S	RT-LAMP	F/NE	[66]
DETECTR	NP/OP	Conventional	Cas12a	E and N	RT-LAMP	LFA	[58]
RCSMS	Saliva	TI	Cas12a	E and N	RT-LAMP	LFA	[62]
STOPCovid.v2	NP	Magnetic Bead Assisted	AapCas12b	N	RT-LAMP	F/LFA	[55]
AIOD-CRISPR	-	-	Cas12a	N	RPA	F/NE	[63]
CASdetec	-	-	Cas12b	RdRP	RT-RAA	F	[64]
CRISPR-FDS on Chip Assay	Saliva	Optimized Lysis	Cas12a	Orf1ab	RT-RPA	F-SP	[67]
CRISPR-COVID	NP/OP	Conventional	Cas13a	Orf1ab	RT-RPA	F	[74]
SHERLOCK	NP/OP	Conventional	Cas13a	Orf1ab/S	RT-RPA	LFA	[70]
SHINE	NP/OP	TI	Cas13a	Orf1a	RT-RPA	F/LFA	[73]
Fozouni et al.	-	-	Cas13a	E and N	None	F-SP	[87]

There was no clinical validation performed for AIOD-CRISPR, CASdetec, and Fozouni et al. Therefore, marked with a "-". Diagnostic tests that employ thermochemical Inactivation (SHINE, RCSMS) and optimized lysis (CRISPR-FDS on Chip Assay) for viral nucleic acid extraction do not require RNA isolation. E: Envelope Gene, F: Quenched Fluorescent Reporter, LFA: Lateral Flow Assay, N: Nucleocapsid gene, NE: Naked Eye, NP: Nasopharyngeal Swab NS: Not Specified, Orf1ab: Open Reading Frame 1ab Polyprotein, OP: Oropharyngeal Swab, RdRP: RNA dependent RNA Polymerase, RT-LAMP: Reverse Transcription loop-mediated amplification, RPA: Recombinase Polymerase Amplification, RAA: Recombinase Aided Amplification, S: Spike gene SP: Smart Phone, TI: Thermochemical Inactivation

Currently, U.S. CDC recommends nasopharyngeal specimen collection, and oropharyngeal specimens are also considered acceptable specimens [76]. This collection method may impose a risk on the healthcare worker due to direct exposure. However, please note that there is limited data regarding aerosol generation and risk of transmission during NP/OP specimen collection procedures [77].

Saliva samples offer practical and logistical advantages for the diagnostic efforts as they can be directly collected by the patient. To compare the relative diagnostic value of nasopharyngeal and saliva samples in early infection, non-human primates were infected with SARS-CoV-2 in a study [67]. Results showed that the mean SARS-CoV-2 levels (detected with CRISPR-FDS) were substantially higher and more stable for a long time in oropharyngeal saliva samples compared to nasal swabs [67]. Another study indicates that posterior oropharyngeal saliva samples had better positive percent agreement than nasopharyngeal samples [78]. There is an anecdotal example of a leukemia patient, whose nasopharyngeal swab was tested negative with RT-qPCR but positive results were obtained from saliva with the CRISPR-FDS test [67,79]. In a study, saliva samples were demonstrated to have superior sensitivity over nasopharyngeal swabs [57]. Altogether, these studies suggest that saliva can be an acceptable alternative to nasal swabs.

RNA Isolation

The majority of the nucleic acid detection methods for COVID19 require a prior RNA isolation step. Isolation of viral RNA requires commercial kits, expensive reagents, and long processing times [62]. Therefore, simpler methods for RNA isolation could be more appropriate for a point-of-care diagnostic test. There is some alternative simple to perform methods to isolate RNA. One of these methods makes use of magnetic beads [55]. Some of the CRISPR protocols do not require prior RNA isolation. These approaches consist of CRISPR-FDS, RCSMS, and SHINE. These protocols are made possible by optimizing the lysis step and thermochemical inactivation of RNases.

Amplification Methods

Isothermal amplification methods have many advantages over the conventional PCR method in terms of simplicity, rapidity, and low cost [63]. Recombinase polymerase amplification (RPA) [80] and loop-mediated isothermal amplification (LAMP), are the mainly preferred methods used in many point-of-care diagnostic tests as they don't require a thermocycler and take a shorter time. Furthermore, buffers of these amplification methods can be optimized with Cas enzymes [81] to be used in a single tube which removes the requirement of sample transfer and decrease the contamination risk. Despite their advantages, there are currently some challenges in application

such as false-positive results due to non-specific amplification [82,83].

Recombinase polymerase amplification (RPA) operates at the temperature of 37-42°C, which is an advantage over LAMP because it does not need any instrumentation for the amplification. Even holding the tube in the hand could be enough to proceed. The disadvantage of RPA is that it has support chain restrictions whereas requisite enzymes for LAMP are more readily available. Loop-mediated isothermal amplification operates at 55-70°C. Therefore, a thermostable Cas enzyme like AapCas12b must be used to be able to reduce the test into a single tube [55]. The required temperature can be provided with a heat block or even a simple sous-vide cooker. RT-LAMP can be severely inhibited by saliva, thus requiring a prior RNA isolation step or a well-optimized step for the inactivation of salivary enzymes. Some COVID19 point-of-care diagnostic approaches use RT-LAMP such as COVID19 Penn-RAMP [84], HP-LAMP [85] and a colorimetric assay [86]. But it is suggested that using CRISPR-Cas12 improves the sensitivity and specificity of the tests compared to using only RT-LAMP [66].

Besides these amplification methods, there is a CRISPR-based approach that does not require nucleic acid amplification. It was previously reported that using different crRNAs together can increase the sensitivity of the test but Fozouni et al. demonstrated that multiple crRNAs can overcome the need for nucleic acid amplification [87]. They devised a protocol that can detect the SARS-CoV-2 genome quantitatively in pre-extracted RNA samples under 30 minutes. Test reached the sensitivity of 100 copies/µL.

Readout

There are mainly two approaches to detect amplicon in CRISPR-based COVID19 tests: Lateral flow assay and fluorophore quencher paired ssDNA/ssRNA. Lateral flow assay can be easily found anywhere as a paper dipstick, and it is very easy to apply. It can also be used in serological tests [88]. As it can be seen from Figure 1, there are two lines on the paper dipstick. The control line has streptavidin and if the FAM-biotin reporter is not cleaved, it is stuck in the control line due to biotin-streptavidin reaction. Whereas, if the Cas enzyme gets activated and cleaves the reporter, free FAM can reach the second line and be kept in place by antibodies specific for FAM. The important advantage of lateral flow assay over fluorophore signal is that it provides a simple, naked eye readout that does not require special instrumentation. It provides a simple qualitative result such as positive or negative. However, this qualitative result might be considered as a disadvantage as we will not be able to detect the viral titer and understand the condition of the patient. Opening the tube for lateral flow assay also brings a contamination risk. This contamination risk may prohibit its use outside a laboratory environment. Therefore, detection of fluorescence signals might be better in terms of contamination risk. A fluorescence readout could detect the viral load of patient samples in real-time. This quantitative information gives us the chance to observe the natural course of the disease and intervene accordingly. Detection of the fluorescence signal requires special instrumentation but it is demonstrated in many studies that a mobile phone can be used to detect the fluorescent signal [67,87]. Therefore, this requirement does not restrict its application as a point-of-care diagnostic test. Further, it is reported in a study that researchers received better signals (less background noise) with a mobile phone compared to the laboratory instrument [87]. The fluorescence signal can also be visualized with a blue LED and become observable without a device [66].

Cost

The cost of a point-of-care diagnostic test holds great importance since an expensive test has limited applicability. The cost of the RT-qPCR test may range from $25 to 100 per test and immunological tests cost around $6-8 per test [20]. Whereas, material costs of CRISPR-COVID were reported to be less than $3,5 on research scale [74]. It was previously reported that a SHERLOCK test can be redesigned and synthesized for as low as $0,61 per test [68]. Therefore, in terms of cost, CRISPR-based diagnostic tests are valuable candidates as point-of-care diagnostic tests.

Concluding Remarks and Future Perspective

CRISPR-based diagnostics have many advantages as emphasized throughout the article. They are very easy to perform and will become easier when these optimized reagents become commercially available. They do not require complex equipment or trained personnel. Required pieces of equipment are a pipette, a reaction tube, reagents, a heat block, and a mobile phone (or a paper dipstick). These types of equipment can be found anywhere. Therefore, it can be used widely in rural areas and at the bedside without referring to a central laboratory. Being independent of central laboratories and sample transportation would reduce the turnaround time substantially. Some of these methods even do not require RNA isolation and some are independent of nucleic acid amplification. This flexibility further decreases the sample-to-answer time (as can be seen in CRISPR-FDS). Obtaining such rapid results provide vital advantages in many cases. In schools, the students and the teachers could be tested before entering school. In airplanes, passengers could be tested before boarding. Therefore, these tests could prevent the spread of the virus more efficiently.

An ideal point of care diagnostic test is inexpensive, easy to perform, has short sample-to-answer time, and high accuracy. Among the reviewed CRISPR-based approaches, we found CRISPR-FDS to be the most suitable one as a point-of-care diagnostic test. Starting from the specimen collection, saliva samples can be collected by the patient himself. Therefore, reducing the extensive protective gear required for the healthcare worker. After specimen collection, instead of conventional RNA isolation, an optimized lysis protocol is followed. As a result, expensive reagents and commercial kits are not required for RNA isolation which reduces the cost and turnaround time substantially. The sample-to-answer time of CRISPR-FDS is only 15 minutes (5 minutes lysis and 10-minute RT-RPA amplification followed by a smartphone-based readout). Therefore, its sample-to-answer time occurs to be the shortest among the nucleic acid detection methods. The test yielded the limit of detection as 0,38 copies/µL, which is the second-best result after STOPCovid.v2. Swab samples of 103 patients that were tested with CRISPR-FDS exhibited complete concordance with RT-qPCR results and saliva samples exhibited a 1,3% false-negative rate. Overall, we found CRISPR-FDS to be the best CRISPR-based approach for the diagnosis of COVID19 in terms of turnaround time, the limit of detection, sensitivity, and specificity. Other approaches also show promising results. In terms of clinical validation, SHERLOCK, STOPCovid, and DETECTR were the most extensively studied ones and they exhibited high sensitivity and specificity compared to RT-qPCR.

Recently, worrisome SARS-CoV-2 variants have emerged [89-91]. Currently available vaccines are thought to be effective against these variants [92-95]. However, a new variant can bypass the vaccination immunity and we should be prepared for such an event. CRISPR-based diagnostics share the same restrictions with RT-qPCR in terms of new variants. If there is a mutation in the primer binding sites or crRNA binding sites, both tests could fail to give accurate results. The important advantage of CRISPR-based diagnostics is that multiple different crRNAs can be efficiently combined and multiplexed [96]. That way, if there is a mutation in one of the crRNA binding sites, the difference between the obtained and expected fluorescence signal could alarm us when we face a new variant [87]. Next-generation sequencing will remain to be the golden standard for the identification of new variants/species [97] but CRISPR-based diagnostics might be helpful for screening. After the identification of a new variant, a specific crRNA could be easily designed and added to the protocol. As shown in the study of Fozouni et al., the combination of multiple crRNAs covered 4115/4118 of the genomic variants in the database [87]. The flexibility to design and use multiple crRNAs is regarded as one of the biggest advantages of CRISPR-based diagnostics.

To conclude, CRISPR-based diagnostics hold great potential as a next-generation point-of-care diagnostic test for COVID19, as well as many other infectious diseases like Zika virus, Dengue virus [68], Human Immunodeficiency Virus (HIV), and Mycobacterium Tuberculosis [53]. We have learned the importance of fast and accurate diagnosis during the pandemic and now we should focus on how to improve these tests further in terms of cost and widespread availability. RT-PCR's restrictions

are not just technical but also financial. Even though high-frequency testing with RT-PCR can help to control the spread of infectious disease, it has a significant economic burden on low-middle income countries. We have learned that worldwide precautions (instead of country-wide) are necessary to contain/prevent a pandemic. Every country should be prepared for novel variants/species and these tests may provide low-middle income countries a better ground to fight against infectious diseases. Hopefully, these tests may prevent another infectious disease or a new variant from becoming a pandemic.

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Author contribution

Study conception and design: İAU and PD; data collection: İAU; analysis and interpretation of results: İAU and PD; draft manuscript preparation: İAU. All authors reviewed the results and approved the final version of the manuscript.

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Ethical approval was not required for the article.

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The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Investigation of Mean Platelet Volume as a Prognostic Criterion in Non-Healing Wounds

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ABSTRACT

Objective: We aimed to evaluate if the Mean Platelet Volume (MPV) is an acute phase reactant in non-healing wounds, by analyzing its correlation with Erythrocyte Sedimentation Rate (ESR).

Method: Our study was carried out in a descriptive type with the participation of patients with non-healing wounds. The laboratory data and characteristics of the patients were accessed retrospectively, and the obtained data were recorded in the data recording form.

Results: The sample group consisted of 92 patients with non-healing wounds. 26.9% of the patients with non-healing wounds had pressure sores, 37.6% of them had diabetic foot wounds, 18.3% had non-healing wounds developed after trauma, and 17.2% had necrotizing fasciitis. The average age of the patients was 53.22±19.13, and the average length of stay in the hospital was 108.98±18.78 (min 3 months, max 6 months) days. The MPV value, which was found to be high in the early stages of non-healing wounds, decreased after the wound was completely healed. When the MPV value was compared to ESR, an acute phase reactant, a positive and strong statistically significant correlation was found between MPV and ESR based on the result of this correlation analysis ($r=0.256$, $p<0.01$).

Conclusion: MPV can be used as a marker, just like ESR, in the presence of non-healing wounds. MPV value can be measured with blood taken into the complete blood count. However, an extra blood sample and a different tube are required for ESR. Using MPV value instead of ESR will provide savings in terms of cost and labor.

Keywords: Mean platelet volume, non-healing wounds, prognostic criteria

INTRODUCTION

Wound healing includes complex biochemical and cellular processes, and it is affected by many variables such as intracellular components and extracellular matrix [1,2]. In non-healing wounds, the process of new vessel formation (angiogenesis) is often impaired. Due to insufficient blood supply, nutrients and oxygen cannot be transported to the wound [3-5]. Non-healing wounds cause low comfort levels, low quality of life and low morale and motivation, loss of work power, loss of function due to the wound, long morbidity period and/or risk of death. This reveals the importance of the issue [1,5]. In addition, depending on the location and size of the wound, periodic maintenance often causes the individual to be unable to perform self-care alone, and this is emotionally and socio-economically exhausting. If non-healing wounds deepen over time and progress towards the bone, they cause osteomyelitis. Osteomyelitis, which can be defined as bone inflammation, prepares the

ground for amputation. Despite the high awareness of the problems caused by non-healing wounds, its management cannot be provided at an optimal level.

Platelets are small and morphotic elements of blood. Platelets are effective at the stage of hemostasis and fibrosis in the optimally smooth and expected wound healing process. In the first stage of the wound healing process, platelets accumulate at the wound site. When antagonists (Thromboxane A2 receptor antagonists, etc.) are activated, cytoplasmic granular content and inflammatory cytokines (interleukin-1 and interleukin-6, and tumor necrosis factor-alpha) are released and aggregated. Thus, fibrosis and inflammatory processes are initiated [6,7]. Platelets play an important role in the pathogenesis of local and systemic inflammation. Interleukin (IL)-6, which increases in the inflammatory process, has a direct effect on megakaryocytes and stimulates thrombopoietin simultaneously [8].

Mean Platelet Volume (MPV) can provide important data on cardiovascular and respiratory diseases [9-12], rheumatoid arthritis [13], juvenile systemic lupus erythematosus [14], neoplasms [15,16], diabetes mellitus [17] and Crohn's disease [18,19]. The MPV value may change in such systemic inflammations. A typical mean value of platelet volume ranges from 9.7fL to 12.8 fL in intravascular fluid. In the literature reviews, it was reported that MPV value increases in the presence of ischemic heart disease, stroke, venous thromboembolism, hypertension, metabolic syndrome and neoplasm [20-26] There are also studies showing that MPV value decreases in the presence of trauma and sepsis [6,27]. Normally, the MPV value is in the range of 7.5 fL and 10 fL.

In case of bone damage caused by non-healing wounds, magnetic resonance imaging (MRI) and probe-to-bone (PTB) tests can be applied. Erythrocyte Sedimentation Rate (ESR) guides the applications of care and treatment in all non-healing wounds. Apart from the cost of MRI and PTB, they fall short of the reliability of ESR in terms of the certainty of their results [28]. ESR is defined as the sedimentation rate of erythrocytes. In the presence of inflammation in the body, some protein structures (fibrinogen, α2, β and γ globulin) combine with erythrocytes, causing erythrocytes to precipitate faster [29].

Debris accumulation in the wound paves the way for bacteria to multiply. Dead cells and cell debris in necrotic tissue reduce host defenses and promote infection. The presence of necrotic tissue creates a mechanical obstacle to wound healing, limiting the epithelization of the wound surface, prolonging the inflammatory process and causing inflammation [1-5]. This creates a non-healing wound.

Acute phase reactants (AFRs) are proteins whose serum concentrations increase (positive AFR) or decrease (negative AFR) by at least 25% in response to inflammation. Positive AFRs include Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), procalcitonin (PCT), serum amyloid A (SAA), ferritin, and so forth. Among these, CRP, PCT and ESR are the most used in the evaluation of infection and inflammation [30]. However, these are laboratory tests that are costly and require a new tube of blood from the patient. It is advantageous to work on MPV with other parameters on complete blood count. In this study, we aimed to investigate the MPV value as a prognostic criterion in non-healing wounds.

MATERIALS AND METHODS

The data of our study were analyzed retrospectively. The research design is descriptive. The sample group consisted of 92 patients with non-healing wounds who were followed up in the Plastic, Reconstructive and Aesthetic Surgery Clinic of Adıyaman University Medical Faculty Hospital between 2014 and 2019. Patients treated in the study were included in the sampling. The purposive sampling method was used in sampling.

Inclusion Criteria;

i. Patients with a non-healing wound
ii. Being treated in the specified clinic between 2014 and 2019
iii. Being 14 years old or older
iv. Patients without malignancy

Exclusion Criteria;

i. Non-problematic, acute wounds
ii. Patients followed up outside the period of 2014 and 2019
iii. Being under the age of 14
iv. Patients with malignancy

Patient data were analyzed retrospectively using past computer-based records, patient files and electronic health records, and these data were recorded in a data record form developed by the researcher. Parameters such as sociodemographic characteristics, wound etiology, length of hospital stay, blood values of the patients were recorded.

Statistics

Statistical analysis of the results obtained in the study was performed using IBM SPSS (Statistical Package for the Social Sciences) Statistics 25. Descriptive statistical methods (Frequency, Standard Deviation, Arithmetic Mean) were used in data evaluation. The Kruskal-Wallis test, Wilcoxon signed-rank test, One-way ANOVA and Post hoc test were performed to determine the statistical significance of the differences between the means of the groups. Correlation analysis was used. Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to find normal distribution assumptions. The results were evaluated at 95% confidence interval with a significance level of $p<0.05$.

Ethics

Prior to the study, the requisite approvals were obtained from Adıyaman University Clinical Research Ethics Committee (Decision No:2015/02-05). After the Ethics Committee Report was issued, access to patient records was provided and patient confidentiality was taken into account, and only health records were accessed, identity information was not examined.

RESULTS

The sample group of this study consisted of 92 patients with non-healing wounds. Non-healing wounds were classified as follows; 26.9% (n=25) of the patients had pressure sores, 37.6% (n=35) of them had diabetic foot wounds, 18.3% (n=17) had non-healing wounds developed after trauma, and 17.2% (n=16) had necrotizing fasciitis. When the characteristics of the patients were examined, the average age of the patients was found as 53.22±19.13 (min 14, max 80) years. 60.2% (n=56) of the sample group was determined as male and 39.8% (n=37) as female. The average length of stay in the hospital was determined as 108.98±18.78 days (min 3 months, max 6 months). The patients were discharged after the recovery of the non-healing wounds. The average healing time of problematic wounds was determined as 114±24.46 days (min 3 months, max 6 months). Laboratory data of the patients are included in Table 1.

Average blood values were determined as follows; Phosphorus as 3.36±0.93 (min 1.9, max 7.4), Calcium as 9.03±0.53 (min7.5, max 10.1), Vitamin D as 12.57±11.86 (min 2.49 max, 103.16), Parathyroid hormone as 42.23±33.69 (min 12.6, max 218.3), Magnesium as 2.19±0.42 (min 1.23, 3.23), ESR at the time of admission as 44.15±23.3 (min 2, max 113), WBC at the time of admission as 10.04±4.08 (min 3.83, max 22.77), MPV at the time of admission as 10.52±3.72 (min 4.54, max 13.6), PLT at the time of admission as 287.05±89.01 (min 139.5, max 610.3), HGB at the time of admission as 12.11±1.74 (min 8.81, max 18.3), Albumin as 3.14±0.52 (min 1.6, max 4.24), total protein value as 6.69±0.72 (min 4.6, max 8.2) and ALP as 92.48±57.43 (min 18, max 405) (Table 2.).

According to the Wilcoxon analysis, there is a statistically significant relationship between admission and discharge values of the means of HGB, WBC, ESR and MPW. Accordingly, while the ESR value was 44.15±23.3 (min 2, max 113) at the time of admission, it was determined as 26.49±15.51 (min 6, max 83) at the time of discharge (p=0.001), and while the MPV value was 10.52±3.72 (min 4.54, max 13.60) at the time of admission, it was determined as 6.9±1.65 (min 4.4, max 14.83, median 6.62) at the time of discharge (p=0.024).

Table 1. Results of Patients' Laboratory Data.

Laboratory Data	(Mean±SD)	Min-Max
Phosphor	3.36±0.93	1.9-7.4
Calcium	9.03±0.53	7.5-10.1
Vitamin D	12.57±11.86	2.49-103.16
Parathormone	42.23±33.69	12.6-218.3
Magnesium	2.19±0.42	1.23-3.23
ESR (Admission)	44.15±23.3	2-113
WBC (Admission)	10.04±4.08	3.83-22.77
MPV (Admission)	10.52±3.72	4.54-13.6
PLT (Admission)	287.05±89.01	139.5-610.3
HGB (Admission)	12.11±1.74	8.81-18.3
Albumin	3.14±0.52	1.6-4.24
Total Protein	6.69±0.72	4.6-8.21
ALP	92.48±57.43	18-405

Table 2. Comparison of Laboratory Data and Values of Non-Healing Wounds at the time of Admission and Discharge.

Laboratory Data		Value at the time of Admission	Value at the time of Discharge	Items
HGB	Mean±SD	12.11±1.74	12.32±1.79	p=0.158
	Min-Max (Median)	8.81-18.3	8.74-17.87	z=-1.413
WBC	Mean±SD	10.04±4.08	7.49±1.81	**p=0.001****
	Min-Max (Median)	3.83-22.77	4.25-14.37	z=-6.483
PLT	Mean±SD	287.05±89.01	280.11±84.22	p=0.141
	Min-Max (Median)	139.5-610.3	83.86-576.6	z=-1.472
ESR	Mean±SD	44.15±23.3	26.49±15.51	**p=0.001****
	Min-Max (Median)	2-113	6-83	z=-7.274
MPV	Mean±SD	10.52±3.72	6.9±1.65	**p=0.024***
	Min-Max (Median)	4.54-13.60	4.4-14.83	z=-5.007

*p<0.05, **p<0.01, z; Wilcoxon signed-rank test

Table 3. Comparison of MPV Value with the Type of Non-Healing Wound.

	Type of Non-healing Wound	Admission Value (Mean±SD)	Discharge Value (Mean±SD)	p, z
MPV value	Pressure Sores (1)	10.35±1.40 (min 5.57, max 13.20)	6.32±0.63 (min 5.21, max 7.16	**p=0,001**** z=1654
	Traumatic (2)	10.29±1.3 (min 5.49, max 13.70)	6.46±0.76 (min 4.74- max 8.89)	p=0.56 z=11003
	Diabetic Foot (3)	10.28±1.4 (min 5.65, max 13.50)	6.44±0.71 (min 4.78- max 8.35)	p=0.06 z=2211
	Necrotizing Fasciitis (4)	10.34±1.9 (min 4.54, max 13.80)	6.21±1.48 (min 4.63, max 8.93)	**p=0.001**** z=756
	Post hoc			4>1>2, 3

*p<0.05, **p<0.01, z; Wilcoxon signed-rank test, F=Post hoc test

The comparison of MPV and type of non-healing wound was given in Table 3. The difference between the admission and discharge values of MPV value was found to be statistically significant in necrotizing fasciitis and pressure sores (p<0.01). In the post hoc analysis, the statistical difference between MPV values at the time of admission and discharge in non-healing wounds, from the highest to the lowest, was listed as follows; pressure sores > necrotizing fasciitis > traumatic wound > diabetic foot wound.

In the comparison of sociodemographic data with the types of non-healing wounds, there is a statistically significant difference in the types of wounds depending on gender (p=0.001, t=1214). Decubitus ulcers and traumatic wounds were found to be higher in males than females (p=0.001, KW=11503). The higher rate of the diabetic foot and necrotizing fasciitis in women compared to men was found to be statistically significant (p=0.001, KW=11597).

Table 4 includes the correlation of laboratory data. A positive and strong statistically significant correlation was found between MPV and ESR (r=0.256, p=0.016). There is a positive and moderately significant correlation between Calcium and Vitamin D (r= .457, p=0.000). There is a strong and negative correlation between calcium and ESR at the time of admission (r=-. 216, p=0.043). Similarly, there is a strong and negative correlation between Vitamin D and Parathyroid hormone (r=-. 249, p<0.029).

Table 5 presents the comparison of the laboratory data with the types of non-healing wounds. Accordingly, a statistically significant correlation was found between the types of non-healing wound and vitamin D, parathyroid hormone and magnesium values. 25-hydroxy vitamin D was found to be the highest in non-healing wounds developed after trauma, and the lowest in necrotizing fasciitis (p=0.009, KW=11600). The parathyroid hormone level was found to be the highest in necrotizing fasciitis and the lowest in pressure sores (p=0.037, KW=8466). Magnesium value was found to be the highest in non-healing wounds developed after trauma and the lowest in pressure sores (p=0.001,

Table 4. Correlation of Laboratory Data.

Laboratory Data	r, p	Magnesium	Phosphor	Calcium	25-hydroxy vitamin D	Parathormone	ESR	MPV
Magnesium	r	1.000						
	p	-						
Phosphor	r	-0.06	1					
	p	.580	-					
Calcium	r	0.02	0.206	1				
	p	.852	.051	-				
25-hydroxy vitamin D	r	-0.021	0.211	.457	1.000			
	p	.848	.051	**.000****	-			
Parathormone	r	0	-0.038	-0.071	-.249	1		
	p	0.367	.740	.534	**.029**	-		
ESR	r	0.133	0	-.216	-0.131	-0.131	1	
	p	.221	0.187	**.043***	.233	.251	-	
MPV	r	0.089	0.037	0	-.008	0.065	.256	1.000
	p	.409	.726	0.61	.939	.566	**.016***	-

*p<0.05, **p<0.01, r; Spearman Rank Correlation

KW=16928). In the Post hoc analysis, the HGB value, from the highest to the lowest, was determined as follows; pressure sores> necrotizing fasciitis> traumatic wound> diabetic foot. MPV values were higher in pressure sores and necrotizing fasciitis than the diabetic foot and traumatic wound.

DISCUSSION

The treatment of non-healing wounds aims to reduce morbidity and mortality, to increase the quality of life and comfort, and to provide socioeconomic well-being. In the treatment of non-healing wounds, clinicians usually deal with the wound area. However, the focus should not only be on the wound, but also on laboratory data. In this study, we obtained evidence for evaluating MPV as a prognostic criterion in the presence of problematic wounds. In this study, we obtained evidence for evaluating MPV as a prognostic criterion for non-healing wounds. In our study, the significant difference between MPV values at the time of admission and discharge suggests that MPV may be a prognostic criterion. At the same time, a positive and strong statistically significant relationship (r=0.256, p=0.016) was found between MPV and ESR. This has been a finding that supports our hypothesis. This evidence is promising as it means that MPV can be used instead of ESR.

The sample group of this study consisted of 92 patients with non-healing wounds. Non-healing wounds were classified as follows; 26.9% (n=25) of the patients had pressure sores, 37.6% (n=35) of them had diabetic foot wounds, 18.3% (n=17) had non-healing wounds developed after trauma, and 17.2% (n=16) had necrotizing fasciitis. The average length of stay in the hospital was determined as 108.98±18.78 days (min 3 months, max 6 months). The patients were discharged after the recovery of the non-healing wounds. They were then called to be followed up periodically. The mean treatment period for non-healing wounds was determined as 114±24.46 days (min 3 months, max 6 months).

In the literature, it has been reported that CRP, ESR, platelet and MPV are important biomarkers in determining clinical processes and MPV levels are associated with ESR and CRP levels [31-34]. In a previous study, 83 patients with ulcerative colitis were examined, and in this study, an increase in ESR, CRP, neutrophil levels and a decrease in MPV were found. In addition, the relationship between MPV level and inflammation has been reported in previous studies [35-37]. It is thought that the increasing number and activity of platelets during the inflammation process affects MPV levels [38,39].

Crohn's disease, Hepatitis B, Acute Appendicitis cases are reported to be associated with MPV levels, depending on the inflammation [40,41]. In a previous study, it was reported that the MPV value varied during the remission and relapse periods of inflammatory bowel diseases, increasing during remission and decreasing during relapse periods [42]. Therefore, it is possible that MPV levels may

Table 5. Comparison of the Laboratory Data with the Types of Non-Healing Wound.

Laboratory Data	Types of Non-healing Wound	n	Mean±SD	Min-Max	p, KW, t
Albumin	Pressure Sores	25	3.05±0.62	1.6-4.2	p=0.270 KW=3924
	Traumatic	17	3.36±0.48	2.6-4.2	
	Diabetic Foot	34	3.15±0.5	2.1-4.24	
	Necrotizing Fasciitis	16	3.03±0.41	2.23-3.56	
Total Protein	Pressure Sores	25	6.78±0.56	6.1-8.04	p=0.100 KW=6255
	Traumatic	17	6.46±0.93	4.6-7.6	
	Diabetic Foot	34	6.95±0.58	5.9-8.2	
	Necrotizing Fasciitis	16	6.26±0.76	5.2-6.99	
ALP	Pressure Sores	25	95.68±36.83	49-166	p=0.368 KW=3157
	Traumatic	17	106.18±114.83	36-405	
	Diabetic Foot	34	83.21±22.7	18-121	
	Necrotizing Fasciitis	16	92.63±47.05	57-199	
Phosphor	Pressure Sores	23	3.43±1.16	2-7.4	p=0.928 t=0.111
	Traumatic	17	3.33±0.72	2.1-4.3	
	Diabetic Foot	34	3.29±0.88	1.9-5	
	Necrotizing Fasciitis	16	3.45±0.93	1.9-5.2	
Calcium	Pressure Sores	25	9.08±0.45	7.8-9.8	p=0.962 KW=0.288
	Traumatic	17	8.98±0.71	7.5-9.7	
	Diabetic Foot	34	9.03±0.43	8.4-10.1	
	Necrotizing Fasciitis	16	9.02±0.68	7.9-9.9	
25-hydroxy vitamin D	Pressure Sores	21	14.03±21.26	2.49-103.16	**p=0.009**** KW=11600
	Traumatic	17	17.02±7.1	6-26	
	Diabetic Foot	32	10.51±6.44	2.95-28.06	
	Necrotizing Fasciitis	16	10.06±3.66	4-16	
Parathormone	Pressure Sores	18	29.11±9.42	12.6-46.4	**p=0.037**** KW=8466
	Traumatic	17	41.54±19.99	22-74	
	Diabetic Foot	28	46.01±50.74	18-218.3	
	Necrotizing Fasciitis	16	51.13±20.53	23-75	
Magnesium	Pressure Sores	22	2.01±0.31	1.23-2.6	**p=0.001**** KW=16928
	Traumatic	17	2.46±0.39	1.9-3.1	
	Diabetic Foot	33	2.09±0.42	1.39-3.23	
	Necrotizing Fasciitis	16	2.34±0.38	1.8-3.1	
HGB (Admission)	Pressure Sores (1)	25	12.73±2.16	9.94-18.3	p=0.104 F=2114 Post hoc; 1>2>3>4
	Traumatic (2)	17	12.26±1.61	8.81-15.6	
	Diabetic Foot (3)	35	11.92±1.41	9.35-15.44	
	Necrotizing Fasciitis (4)	16	11.43±1.64	9.26-14.3	
WBC (Admission)	Pressure Sores	25	10.68±4.44	4.46-22.75	p=0.415 KW=2.851
	Traumatic	17	9.45±4.46	4.26-18.6	
	Diabetic Foot	35	10.53±3.87	6.52-22.77	
	Necrotizing Fasciitis	16	8.57±3.37	3.83-13.57	
PLT (Admission)	Pressure Sores	25	310.34±126.91	139.5-610.3	p=0.293 KW=3724
	Traumatic	17	289.22±42.85	232-345.7	
	Diabetic Foot	35	279.43±75	154.2-515	
	Necrotizing Fasciitis	16	265.02±80.93	193-436.5	
ERS (Admission)	Pressure Sores	23	36.09±20.43	2-88	p=0.198 KW=4222
	Traumatic	17	50.24±30.23	13-113	
	Diabetic Foot	32	45.59±20.76	13-112	
	Necrotizing Fasciitis	16	46.38±22.81	23-89	
MPV (Admission)	Pressure Sores (1)	25	10.35±1.40	5.57-13.20	p=0.907 F=0.904 Post hoc; 1,4>2,3
	Traumatic (2)	17	10.29±1.3	5.49-13.70	
	Diabetic Foot (3)	35	10.28±1.4	5.65-13.5	
	Necrotizing Fasciitis (4)	16	10.34±1.9	4.54-13.8	

*p<0.05, **p<0.01, KW; Kruskal-Wallis Test t; One-Way ANOVA Test, F=Post hoc Test

increase or decrease in systemic inflammations. The increase in MPV in the inflammation is related to the stimulation of thrombopoietin formation by IL-6 and the direct effect of this cytokine on megakaryocytes [43]. MPV acts as an inflammatory marker in many chronic diseases. Thus, it can show the activity of the diseases and the effectiveness of the treatment [44]. In a study investigating the MPV value in diabetic foot wounds, it was found that amputation surgery was more common in patients with high MPV value compared to those with lower MPV value [45].

In our study, the MPV value at the time of admission was found to be high in all four different wound types, including the diabetic foot wound. In addition, when comparing the MPV with the types of non-healing wounds, the difference between the MPV value at the time of admission and discharge was found to be the highest in necrotizing fasciitis and the lowest in diabetic foot wound. The difference between values at the time of admission and discharge was statistically significant in necrotizing fasciitis and pressure sores. In addition, in our study, the ESR value was found 44.15±23.3 at the time of admission and 26.49± 5.51 at the time of discharge (p=0.001). Accordingly, the MPV value was 7.52±1.72 at the time of admission and 6.9±1.65 at the time of discharge (p=0.024). As a prognostic criterion in inflammatory diseases, ESR is an important biomarker, and it increases as a result of inflammatory conditions [46]. In this way, it may be possible to use the ESR value alone for diagnosis because some parameters such as age, gender and the presence of a concomitant secondary infection may also affect the level of ESR. ESR reacts slowly in an inflammatory situation [47]. However, in our study, there is a more remarkable decrease in MPV value compared to ESR. This result can be taken into account as a finding that increases the potency of MPV to be a prognostic criterion.

The data regarding MPV in the literature differ due to the fact that the studies have been conducted in different and independent groups, the reliability of the measurement devices is not known exactly, and the changing approaches of the scientists. In the literature review, no study was found to determine the correlation between non-healing wounds and MPV level. In this regard, our study results can be a basis for the use of MPV as an acute phase reactant in clinical applications in non-healing wounds, as well as guiding clinicians. This study was limited by the nature of its single-center design.

CONCLUSION

In our study, it was found that the MPV value was high in the non-healing wounds prior to the treatment and decreased after the non-healing wounds started to heal. In the literature, no study was found to determine the correlation between ESR and MPV in non-healing wounds. In this regard, this study can be seen as an important source of information. However, some important parameters that may affect MPV could not be evaluated due to the retrospective design of our study. These can be listed as environmental factors, nutrition, alcohol use, smoking and psychological factors.

According to our results, high MPV and ESR values at the same time and a decrease in both values after the healing show the correlation between MPV and ESR levels. Therefore, we recommend that this change in MPV level should be evaluated in larger sample groups. The return of these values to normal after the non-healing wounds are healed is the most important evidence that they increase due to inflammation. We believe that our study will guide clinicians and academics and form a basis for future studies. In addition, if the evidence we have obtained is strengthened, the use of MPV value instead of ESR will save in terms of cost and labor.

Author contribution

Study conception and design: FNT, FD, and AV; data collection: FNT, FD, and AV; analysis and interpretation of results: FNT and FD; draft manuscript preparation FNT, FD, and AV. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Adıyaman University (Protocol no. 02-5/2015).

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Publication Status of Urology Theses in Turkey

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ABSTRACT

Objective: Production of a thesis during residency requires a great deal of dedication and effort. It is an honor to share the results of this effort with everyone when the thesis is published. In this study, we aimed to investigate the factors affecting the publication of a thesis in the field of urology.

Methods: Theses completed between 2014 and 2018 were searched in the Institution of Higher Education Thesis Center. Keywords, title, and authors on the thesis were searched in the PubMed and Google Scholar databases. Journal tags were categorized according to whether they are indexed in Medline or not. Publication status was analyzed with the subject of the thesis, the year of thesis was completed, hospital where urology residents graduated, and the current workplace of the urologists.

Results: Three hundred and fifty-three theses were analyzed in this study. The number of theses that were published in index journals and non-index journals was 65 (18.4) and 15 (4.2%), respectively. The median citation for published theses was 2 (0-21). The subject of the thesis, time passed after the thesis, and the current workplace of urologist was found to be statistically significant in the publication status of the theses ($p<0.001$, $p= 0.02$, $p<0.001$, respectively).

Conclusion: Most of the theses produced by urologists were not published. Published theses received few citations. Theses produced from animal studies and a long period passed over the thesis increase the rate of publication. Urologists whose theses have been published mostly work in tertiary care hospitals.

Keywords: Publication, thesis, citation, urology, index

INTRODUCTION

In most countries, completion of a thesis before graduation from medical school is mandatory. In Turkey, the completion of the thesis is necessary for postgraduate physicians before specializing in their field. It is not only mandatory to complete their specialization in their field but also upgrades their skill in the production of hypothesis, analyzing and interpreting of data, and comparing these results with current literature [1].

The academic productivity of residents can be considered as a quality of center to compose the next generation of scientists. For this purpose, academic staff works as a supervisor with residents to produce a scientifically valid thesis. The process from finding a topic to the completion of the thesis is one of the most challenging processes as it needs time and effort for research assistants as well as urology residents. Research skills in this process are not only to introduce an investigation but also to make residents ready with the experience to assess evidence-based medicine before applying it in their future life.

The publication of the thesis, which is the end-point of this tough process, in indexed journals

and citations of this publication in higher impact journals make the thesis more valuable. Additionally, the published thesis is also important in the future academic life of the urologist in Turkey.

Previously, there have been studies from other disciplines and even urology investigating the publication rate of theses [2-4]. However, in our study, all hospitals providing residency training were taken into consideration, and to the best of our knowledge, this is the first study to investigate the importance of places where urologists work currently in the case publication of theses. Furthermore, in the present study, we aimed to investigate the factors affecting the publication rate of thesis produced in the field of urology.

MATERIALS AND METHODS

This study was approved by the institution ethics review committee by providing the decision/protocol number of 2020/224 in November 3rd, 2020. Theses that have been completed in the field of urology between 2014 and 2018 in Turkey were searched in the Electronic Archive of Higher Education Council Dissertation Center (http://tez.yok.gov.tr/UlusalTezMerkezi/) database by using the terms ''urology'' or ''üroloji''.

It is thought that publication and citation of a thesis need approximately two years after the completion of theses (4). So, theses completed after the year of 2018 were not included in this study. Overall, three hundred and fifty-three theses were evaluated in this study. The author's name, institution's name, the place where the residents graduated, and the subject and the completion year of the thesis were recorded.

Subjects of the thesis were categorized into clinical or experimental studies. Clinical studies are also subcategorized into oncology, urolithiasis, andrology and infertility, functional urology, pediatric urology, female urology, and transplantation, and others. The institutions of urology residents graduated were categorized into university hospitals and education and research hospitals. Cities of hospitals located that residents graduated were categorized into three-biggest cities which are Istanbul, Ankara, and Izmir, and others. Current workplaces of urologists were categorized into secondary or tertiary care hospitals.

Keywords, title, and authors of the thesis were searched in the PubMed and Google Scholar databases to check the thesis was published or not. Journal tags are categorized according to whether they are indexed in Medline or not by searching in the United States National Library of Medicine catalog. The citation status of published theses was checked from the Google Scholar database. Publication status was analyzed with the subcategory of the subject of the thesis, hospitals where residents graduated, time passed after the thesis was completed, and the current workplace of the urologists.

Statistical Analysis

All statistical analyses were performed using the SPSS 22.0 (IBM Corp, Chicago, USA) software. Kolmogorov-Smirnov test was applied to examine the normality of variables. After the distribution was checked, descriptive statistics were presented as median (minimum-maximum) to define the parameters. Pearson Chi-square or Fisher's exact test were used for categorical variables comparison to assess statistically significant differences between groups. The level of the confidence interval was 95% and $P<.05$ was regarded as statistically significant.

RESULTS

Three hundred and fifty-three theses were analyzed in this study. Two hundred and fifty-nine (73.4%) residents graduated from the university hospital and 168 (47.7%) of residents graduated from hospitals in the three biggest cities. One hundred and thirty-four (40.9%) urologists work in the secondary care state hospitals followed by tertiary care hospitals (39%). The number of theses that are published was 80 (22.7%) in total, 65 of which were in index journals and 15 were in non-index journals. Published theses had a median of 2 (0-21) citations and no difference was found between published thesis in Medline or not (2 vs 2, p=0.30). The number of published thesis completed in 2014, 2015, 2016, 2017 and 2018 were 23 out of 89 (25.8%), 22 out of 70 (31.4%), 14 out of 60 (23.3%), 14 out of 71 (19.7%), and 7 out of 63 (11.1%), respectively (p=0.068). Comparing the publication

status of theses before and after the year 2016, it was found 45 out of 159 (28.3%) versus 35 out of 194 (18%) (p=0.02). The demographics of urologists and features of the theses are summarised in Table 1.

Most of theses were clinical studies, mostly oncology (n=105) followed by urolithiasis (n=78), while 48 (13.6%) of theses were animal studies. The highest publication rate (50%) was in animal studies (24 out of 48) followed by andrology and infertility (36.8%). The citation score per publication according to the subject of thesis was found higher in pediatric urology and functional urology groups but there was no statistically significant difference between subjects of published theses (p=0.52). Publication and citation status of theses illustrated in Figure 1 and summarized in Table 2.

When the factors affecting the publication status were analyzed, the subject of the thesis, year of the thesis completed, and the current workplace of urologist were found to be statistically significant in multivariate analysis (p<0.001, p=0.017, and p<0.001, respectively). In multivariate analysis, the group of animal experiments for the production of the thesis was the most powerful factor in the publication status of the thesis [OR= 4.68 (2.33-9.37)] (Table 3).

DISCUSSION

The main purpose of education is the use of acquired knowledge and skills actively throughout life by transforming them into behavior. Reaching the available information by research-based education model rather than didactic learning and lecture-based pedagogic education helps to keep the information more in mind [5]. To be up to date by following the literature with the help of research-based learning is mandatory for everyone who has completed their specialty training to refresh their post-graduate knowledge in new diagnoses and treatment methods. For this purpose, producing scientific publications by using scientific thinking and research methods from a doctoral thesis should be within the framework of basic education in medical residency. Shaping the future academic life might be started from finding the unique topic of the thesis. Hence, in this study, we emphasize that it should be taken into consideration by urology

Table 1. Demographics of urologists and features of theses.

		n (%)
Hospitals of residents graduated	University hospitals	259 (73.4)
	Education and research hospitals	94 (26.6)
Cities of hospitals located	Three biggest cities*	168 (47.7)
	Others	185 (52.3)
Subjects of thesis	Oncology	105 (29.7)
	Urolithiasis	78 (22.1)
	Animal experiment	48 (13.6)
	Andrology and ınfertility	38 (10.8)
	Functional urology	37 (10.5)
	Pediatric urology	21 (5.9)
	Female urology	13 (3.7)
	Transplantation and others	13 (3.7)
Publication status of thesis	Published in medline indexed journals	65 (18.4)
	Published in non-medline indexed journals	15 (4.2)
	Unpublished	273 (77.3)
Year the thesis completed	2014	89 (25.2)
	2015	70 (19.8)
	2016	60 (17)
	2017	71 (20.1)
	2018	63 (17.8)
Current workplace of urologists***	Secondary care state hospitals	134 (40.9)
	Tertiary care hospitals**	128 (39.0)
	City hospitals	29 (8.8)
	Private clinics	37 (11.3)

Abbreviations: n, number; %, per cent; (min- max), (minumum-maximum). * refers to Istanbul, Ankara, Izmir; ** refers to University Hospitals and Education and Research Hospitals, *** stated for 328 theses.

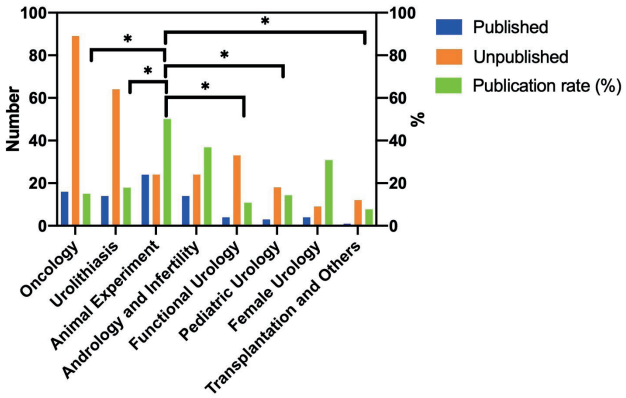

Figure 1. The number of published and unpublished theses, and publication rate according to the the subject of thesis. Chi-square test was used to assess statistically significant differences between groups (* stated for p<0.001).

Table 2. Publication and citation status according to subjects of theses.

		Published n (%)	p	Citation Score median (min-max)	p
Subjects of theses	Oncology (n=105)	16 (15.0)	**<0.001**	2 (0-17)	0.52
	Urolithiasis (n=78)	14 (17.9)		4.5 (0-18)	
	Animal experiment (n=48)	24 (50.0)		2 (0-19)	
	Andrology and infertility (n=38)	14 (36.8)		3 (1-21)	
	Functional urology (n=37)	4 (10.8)		8 (0-16)	
	Pediatric urology (n=21)	3 (14.3)		11 (0-11)	
	Female urology (n=13)	4 (30.8)		1.5 (0-10)	
	Transplantation and others (n=13)	1 (7.7)		0 (0-0)	

Abbreviations: n, number; %, per cent; min-max, minumum-maximum. Chi-square test was used and p<.05 was considered statistically significant and marked in bold.

Table 3. Factors affecting the publication of theses.

		Published n (%)	P	Multivariate Analysis	
				OR (95% CI)	p
Categories of theses	Animal experiments (n=48)	24 (50)	**<0.001**	4.68 (2.33-9.37)	**<0.001**
	Clinical studies (n=305)	56 (18.3)			
Years of theses	2014-2015 (n=159)	45 (28.3)	**0.02**	1.94 (1.12-3.33)	**0.017**
	2016-2018 (n=194)	35 (18.0)			
Hospitals of residents graduated	University hospitals (n=259)	55 (21.2)	0.28		
	Educ and res hospitals (n=94)	25 (26.5)			
Cities of hospitals located	Three biggest cities* (n=168)	41 (24.4)	0.46		
	Others (n=185)	39 (21.0)			
Current workplaces of urologists	Tertiary care hospitals (n=154)	54 (35.0)	**<0.001**	3.30 (1.88-5.79)	**<0.001**
	Others (n=174)	26 (14.9)			

Abbreviations: n, number; %, per cent; OR, odds ratio; CI, confidence interval; Educ and Res; Education and Research. * refers to Istanbul, Ankara, Izmir. Chi-square test was used p <.05 was considered statistically significant and marked in bold.

residents to determine the subject in publication potential as the first step.

The publication rate of thesis in different scientific fields has been investigated in many studies and has been found between 3.5% to 49.7% from nationally and 17.6% to 30% internationally [3,4,6-9]. Ozgen et al. investigated publication patterns of more than twenty-five thousand medical theses between the years 1980 to 2004. The distribution of publication rate in the different fields was from 0.6% to 13.4% as the urology thesis on average (5.7%) in that study [4]. Apart from this study, Yuksel et al. investigated the publication rate of dissertations in the field of urology from medical faculties before the year 2011 [2]. It was found that the overall publication rate was 49.7% and the publication rate in science citation indexed journal was 32.7%. The citation score per publication was between 0.6 to 28 (median citation was 2) according to the subjects of theses. This study included the thesis at least five years after the thesis was completed according to the suggestion of Scherer et al. [10] . In fact, we included the theses at least 2 years after completion and found almost half of the rate of the publications and similar citation score per publication but still comparable to other medical fields published previously [3,11]. However, in our study, a thesis was written 5 years before today more possibility to be published both in univariate and multivariate analysis [(28.3% vs 18%, OR 1.9 (1.1-3.3), p=0.02)].

Both medical students and residents are educated in medical faculties but education and research hospitals only have residents in Turkey. Considering the rate of publication from the thesis in hospital groups and cities residents graduated from, state hospital was found relatively lower rate than the university hospitals and military hospitals and three biggest cities group was found higher rate than the other cities [4]. In our study, we found that there was no difference in hospital groups and cities of residents who graduated. This result which is desirable should show that there is not

any inequality between cities and hospitals where residents graduated from in terms of publication rate.

Publication rate and citation score are affected by subject or type of thesis. Mostly, prospective and experimental animal models have more chance to be published [3,12,13]. In our study, similar to the previous studies, it was seen that there was more publication rate on theses produced from animal-model experimental studies compare to the clinical studies [(50% vs 18%, OR=4.68 (2.33-9.37), p<0.001)]. Although there is a higher publication rate in theses produced from animal studies, there is a discrepancy between citation scores and publication rate in animal studies. In our study, statistically insignificant higher citation scores in theses produced from pediatric urology may be due to the relatively low number of thesis in that group.

The publication also affected by the author's feature (lack of time or ability to use English), subject and method of study (barriers to get ethical approval for prospective clinical studies or negative/unoriginal results), or journals to be submitted (publication fee, lack of funding for open access, and mismatch of the subject of study with the field of the journal [14]. Despite all these limitations and difficulties, it is known that publishing is to share the acquired knowledge about current literature and skills in your field with an academic environment thus serving all these results to the progress of science. This is not only the desire to present their studies to academic life and also to become a part of the academic community which has been supported in the study of Sayek et al. [15] that theses of people with academic career expectations have been published more. The result we obtained in our study was found to be more than three times published theses of people working in tertiary care hospitals compared to those working in secondary care [(35% vs 14.9%, OR=3.3 (1.8-5.7), p<0.001)]. This outcome seems that is a rationale since specialists in academic hospitals tend to publish their work. Our study confirms statistically that if urologists want to work in a tertiary care hospital they should start by producing higher-quality thesis to be published.

We have some limitations in our study. We did not analyze the seniority of consultant effect in the publication rate of theses. Another limitation is that urologists were not investigated whether they were published their thesis before or after they started to the tertiary care hospital.

In conclusion, most of the theses written in the field of urology between 2014 to 2018 were not published. Published theses got relatively low citation scores. Theses produced from experimental studies and a long period passed over the thesis completion increase the rate of publication. To increase the publication rate of theses, the residents should be encouraged to study especially in the animal model experiments under a qualified supervisor for enough time. A great majority of the residents who published their theses have been currently working in a tertiary academic hospitals. The urologist who wants to work in a more comprehensive tertiary care hospital should make an effort to publish his/her thesis.

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Author contribution

Study conception and design: ES and MK; data collection: MK; analysis and interpretation of results: ES; draft manuscript preparation ES. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

This study by decision/protocol number of 2020/224 was approved by the institution ethics review committee and was performed in accordance with the ethical standards of the Declaration of Helsinki.

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Conflict of interest

The authors declare that there is no conflict of interest.

Availability of data and material

The data that support the findings of this study are available from the corresponding author, E.S. upon reasonable request.

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acta medica

ORIGINAL ARTICLE

Evaluation of Sleep Quality with Use of Angiotensin Receptor Neprilysin Inhibitor in Patients with Reduced Ejection Fraction Heart Failure

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ABSTRACT

Background and Aim: It is known that chronic heart failure reduces sleep quality by causing sleep problems. In recent years, it has been observed that sacubitril-valsartan, which is an angiotensin receptor neprilysin inhibitor, reduces mortality and hospitalization in patients with heart failure. The aim of our study is to examine whether sacubitril-valsartan affects sleep quality in patients with reduced ejection fraction heart failure apart from these benefits.

Material and Method: In our study, 44 patients with a history of heart failure with reduced ejection fraction applied to our cardiology outpatient clinic of Gazi Yaşargil Training and Research Hospital were included. Demographic, clinical, laboratory, electrocardiographic, and echocardiographic parameters of these patients were examined. Sacubitril-valsartan treatment was initiated in all patients. Dose titration was performed in patients who could tolerate the treatment. Pittsburgh sleep quality index questionnaire was performed in all patients before treatment and at the end of the second month.

Result: The median age of the study population was 61.5 (47.2 - 70.7, IQR) years and 30 (68.2 %) of them were male. There were 30 (72.7 %) ischemic heart failure patients and 14 (27.3 %) non-ischemic heart failure patients. There was a significant decrease in the number of patients with poor sleep quality after angiotensin receptor neprilysin inhibitor treatment compared to baseline [36 (81.8 %) vs 30 (68.2 %), p= 0.031]. In addition, there was a significant decrease in the total Pittsburgh sleep quality index score of patients compared to the baseline [9.0 (7.0 - 12.0) vs 7.0 (5.0 - 9.0), $p < 0.001$].

Conclusion: In our study, we observed that sacubitril-valsartan treatment improves sleep quality in patients with reduced ejection fraction heart failure.

Keywords: Sacubitril-valsartan, heart failure, sleep quality

INTRODUCTION

Heart failure (HF), which a chronic and progressive disease, is a rapidly increasing and important health problem characterized by a low quality of life, with high mortality and morbidity rates [1-3]. The prevalence of HF is approximately 1-2% of the adult population in developed countries, rising to ≥ 10 % among people > 70 years of age. A recent study in our country observed that the prevalence of HF is 2.9 %, affecting 1.5 million people along with 3 million people under contiguous risk in the soon [2-4]. In HF patients, sleep quality decreases and sleep problems occur in these patients due to symptoms such as shortness of breath, weakness and fatigue, and limited functional capacity. The prevalence of subjective sleep problems reported in HF patients in the literature is between 60 - 95 % [5-8]. In addition, sleep disorders such as difficulty in initiating and maintaining sleep, insomnia, sleep apnea syndrome, excessive daytime sleepiness have been observed frequently in individuals with HF [9-12]. All these adverse outcomes emphasize the need for a better understanding of the sleep problems experienced by patients with HF.

Sacubitril-valsartan is an angiotensin receptor neprilysin inhibitor (ARNI) combination. In the results of the PARADIGM-HF study, there were significant changes in the medical treatment of heart failure with reduced ejection fraction (HFrEF), and ARNI has been used in clinical practice. The beneficial effects of ARNI with the PARADIGM-HF study are as follows; decrease in symptoms, improvement in New York Heart Association- Functional Capacity (NYHA-FC), increase in the quality of life, improvement in physical examination findings, decrease in N-terminal pro-B type natriuretic peptide/troponin levels, decrease in the need for diuretics and/or additional therapy, decrease in systolic pulmonary artery pressure in echocardiography, increase in EF, decrease in ventricular volumes, improvement in global longitudinal strain, reduction in the need for hospitalization and mortality [13].

Although ARNI has beneficial results shown in HFrEF patients, there are no data in the literature on its effect on sleep in these patients. The aim of our study is to investigate the effect of ARNI on sleep quality before and after treatment in patients with HFrEF.

MATERIALS AND METHODS

In our study, 51 patients with a history of HFrEF (EF ≤ 40 %) who applied to our cardiology outpatient clinic of Gazi Yaşargil Training and Research Hospital were examined between 1 July and 31 October 2019. All of the patients were NYHA - FC Class II and III patients. Patients with symptomatic hypotension, creatinine > 2.5 mg/dL, glomerular filtration rate < 30 ml/min, and potassium > 6 meq / L were excluded from the study. In addition, patients who were treated with continuous positive airway pressure and had cognitive dysfunction were excluded from the study. After the exclusion criteria, 46 patients were included in the study. Demographic, clinical, laboratory, electrocardiography, and echocardiographic parameters of these patients were examined. Sacubitril-valsartan treatment was initiated in each patient. Patients who used angiotensin converting enzyme inhibitors (ACEI) before treatment were started 36 hours after the last dose, and patients who received angiotensin receptor blockers (ARB) were started directly. Dose titration was performed 2-4 weeks later in patients who could tolerate the treatment. During the ARNI treatment, there was no change in the HF treatments of the patients, except for ACEI and ARB. While the study was ongoing, two patients who could not tolerate the drug were excluded from the study. Blood pressure measurements, routine laboratory parameters, electrocardiographic and echocardiographic parameters of all patients were enrolled before and after the treatment. The sleep quality of all patients was assessed by using the Pittsburgh Sleep Quality Index (PSQI) questionnaire before and after treatment. The ethics committee approval required for our study was obtained from the Ethics Committee commission of our hospital.

Sleep Quality Index

All patients were asked to complete a self-report questionnaire (Turkish version of Pittsburgh Sleep Quality Index; PSQI) before and 2 months after ARNI treatment. PSQI consists of a 19-items scale and measures 7 components of sleep quality: Subjective sleep quality, sleep delay, sleep duration, habitual sleep efficiency, sleep disturbances, sleep drug use, and daytime dysfunction. The PSQI

score corresponds to the total of individual scores consisting of 7 components. A PSQI score ≥ of 6 points is considered to be indicative of poor sleep quality. The test has a high diagnostic specificity for detecting clinical sleep disturbance. The reliability and the validity of the Turkish version of this index have been confirmed by Ağargün et al [14].

Statistics

The analysis of the data was carried out using SPSS (Statistical Package for Social Science for Windows)-24 packaged software. The histogram and Shapiro-Wilks test were used to verify the normal distribution of data. The continuous variables were presented as a median interquartile range (IQR) (25-75 %) owing to their non-normal distribution. The categorical variables were expressed as percentages. Wilcoxon tests were used for continuous variables. Mc Nemar test was used to compare categorical variables. The statistical significance level of the obtained data was interpreted with the "p" value. Values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Forty-four patients were included in the study. The median age of the study population was 61.5 (47.2 - 70.7, IQR) and 68.2 % (n=30) of patients were male. There were 30 (72.7 %) ischemic HF patients and 14 (27.3 %) non-ischemic HF patients. Clinical, demographic characteristics and medications of all patients were given in Table 1. Systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), alanine transaminase ($p= 0.011$) were significantly lower after ARNI treatment compared to baseline. Clinical, laboratory and echocardiographic parameters of all patients before and after treatment were given in Table 2.

At the end of two months, there was a significant decrease in the total PSQI score of the patients compared to the baseline [9.0 (7.0 - 12.0) vs 7.0 (5.0 - 9.0), $p < 0.001$]. The initial and after treatment scores of the total PSQI score, which consists of a total of 7 components, are given in Figure 1.

After ARNI treatment, there was a significant increase in the number of patients with good subjective sleep quality (26 vs 12, $p < 0.001$) and sleep latency less than once a week (30 vs 19, $p= 0.003$). In addition, there was a significant decrease in the number of patients with sleep latency 1-2 times a week (21 vs 10, $p= 0.013$). The rate of patients with a poor PSQI score before treatment decreased compared to after treatment (81.8 % vs 68.2 %), and this was statistically significant ($p=0.031$). The frequency distribution of dimensions of the sleep quality is given in Table 3.

Table 1. Clinical, demographic features, and medications of all patients.

	n	%
Gender		
Female	14	31.8
Male	30	68.2
Hypertension	18	40.9
Diabetes mellitus	17	38.6
Coronary artery disease	32	72.7
Hyperlipidemia	19	43.1
Familial history	6	13.6
New York Heart Association Functional Capacity		
II	20	45.4
III	24	54.6
IV	-	-
Cardiac resynchronization therapy/ pacemaker	10	22.7
Smoking	23	52.2
Asetilsalisilic acid	32	72.7
Clopidogrel/ticagrelor	13	29.5
Beta blocker	43	97.7
Ivabradine	14	31.8
Angiotensin converting enzyme inhibitors	31	70.5
Angiotensin receptor blockers	10	22.7
Mineralocorticoid receptor antagonists	25	56.8
Hydrochlorotiazid-indapamide	16	36.3
Loop diuretic	31	70.5

DISCUSSION

HF patients are known to have sleep problems and poor quality sleep. To the best of our knowledge, this study is the first to evaluate sleep quality in patients with chronic systolic HF who were given ARNI treatment. As a result of our study, we can say that with ARNI treatment, the sleep quality of HFrEF patients increased, and these patients sleep better.

Table 2. Comparison of clinical, laboratory, and echocardiographic parameters of all patients before and after treatment.

	Before ARNI treatment	After ARNI treatment	p value
Systolic blood pressure, mm/Hg	120 (110 - 130)	115 (110 - 125)	<0.001
Diastolic blood pressure, mm/Hg	75 (70 - 80)	70 (65 - 75)	<0.001
Heart rate, beat/min	66 (64 - 70)	65 (64 - 70)	0.184
Ejection fraction, %	26.5 (20.0 - 34.7)	30.0 (25.0 - 39.0)	<0.001
Left ventricle diastolic parameter, mm	6.00 (5.62 - 6.77)	5.85 (5.60 - 6.50)	<0.001
Left ventricle systolic parameter, mm	4.90 (4.50 - 5.50)	4.80 (4.40 - 5.40)	<0.001
Urea, mg/dL	37 (30 - 48)	39 (30 - 54)	0.700
Creatinine, mg/dL	1.03 (0.84 - 1.17)	1.02 (0.82 - 1.16)	0.906
Aspartat transaminase, IU/L	22 (16 - 28)	20 (15 - 23)	0.155
Alanine transaminase, IU/L	19 (16 - 30)	19 (15 - 26)	0.011
Sodium, meq/L	138.5 (136.0 - 140.7)	139.0 (137.2-141.7)	0.206
Potassium, meq/L	4.3 (4.1 - 4.6)	4.4 (4.0 - 4.8)	0.653
Calcium, meq/L	9.2 (8.8 - 9.8)	9.1 (8.9 - 9.6)	0.103
White blood cell, 10^9/ L	8.46 (7.06 - 10.31)	8.40 (7.14 - 10.22)	0.824
NLR	2.52 (1.79 - 3.53)	2.62 (2.03 - 3.33)	0.889
Hemoglobin, gr/dL	14.3 (12.1 - 15.2)	14.0 (13.1 - 15.1)	0.262
Platelet, 10^9/ L	239 (187 - 277)	236 (195 - 279)	0.806

Data are expressed as median interquartile range. NLR: Neutrophil/lymphocyte ratio

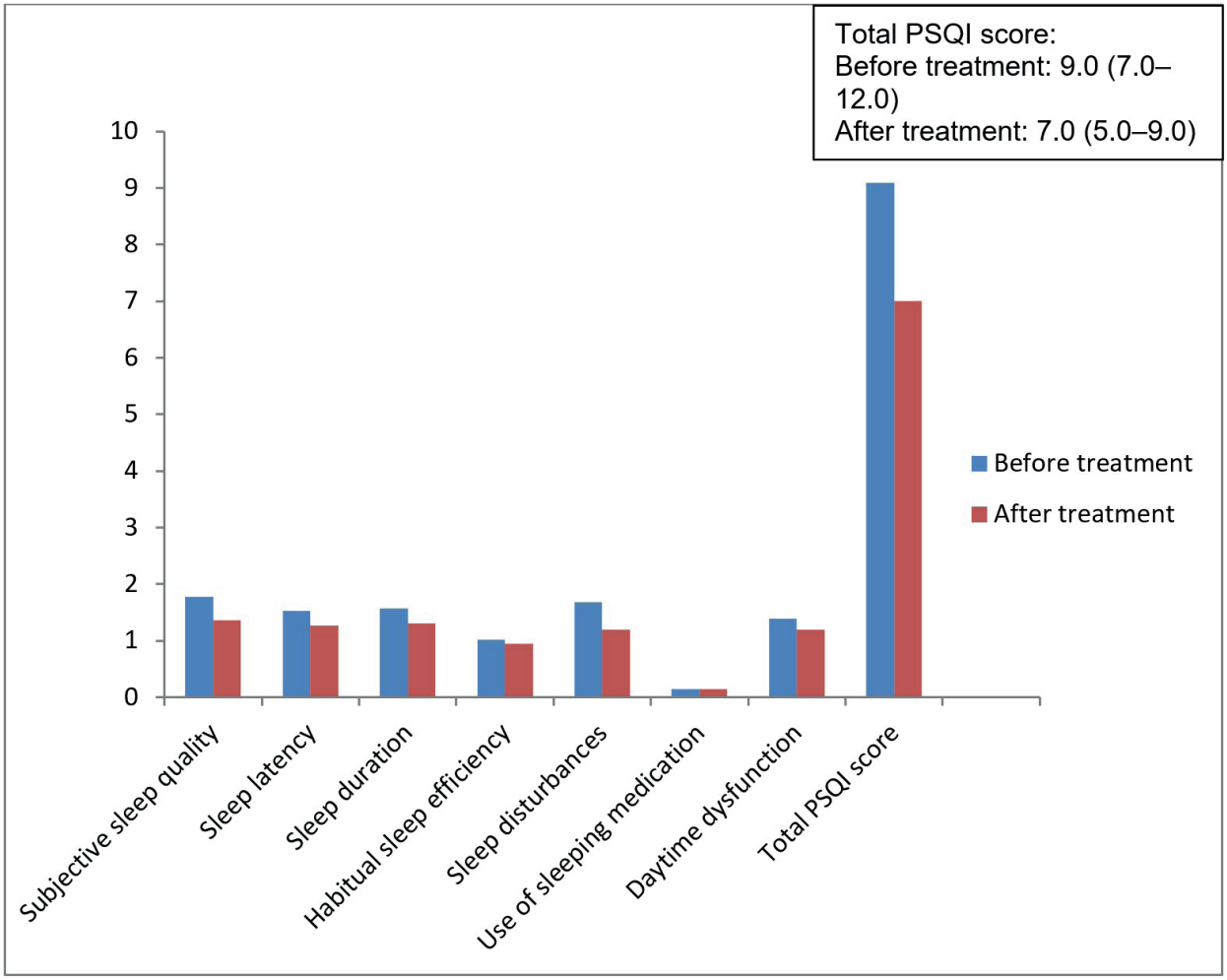

Figure 1. Comparison of the total PSQI score consisting of seven components before and after treatment.

Table 3. Frequency distribution of dimensions of sleep quality.

	Before ARNI treatment	After ARNI treatment	p value
Subjective sleep quality			
Very good, n (%)	3(6.8)	3(6.8)	-
Good, n (%)	12(27.2)	26(59.1)	<0.001
Bad, n (%)	21(47.7)	11(25.0)	0.078
Very bad, n (%)	8(18.2)	3(6.8)	0.125
Sleep latency			
Never, n (%)	2(4.5)	2(4.5)	-
< once/week, n (%)	19(43.2)	30(68.2)	0.003
1-2 times/week, n (%)	21(47.7)	10(22.7)	0.013
≥ 3 times/week, n (%)	2(4.5)	2(4.5)	-
Sleep duration			
> 7 hours, n (%)	4(9.1)	8(18.2)	0.125
6-7 hours, n (%)	18(40.1)	22(50.0)	0.388
5-6 hours, n (%)	15(34.1)	9(20.5)	0.109
< 5 hours, n (%)	7(15.9)	5(11.3)	0.500
Habitual sleep efficiency			
> 85%, n (%)	7(15.9)	9(20.5)	0.500
75-84%, n (%)	29(65.9)	29(65.9)	-
65-74%, n (%)	8(18.2)	6(13.6)	0.502
< 64%, n (%)	0(0)	0(0)	
Use of sleeping medication			
Never, n (%)	41(93.2)	41(93.2)	-
< once/week, n (%)	1(2.3)	1(2.3)	-
1-2 times/week, n (%)	2(4.5)	2(4.5)	-
≥ 3 times/week, n (%)	0(0)	0(0)	-
Total PSQI score ≥ 6, n (%)	36(81.8)	30(68.2)	0.031

HF continues to be a global health problem. It is known that HF patients have negative physical symptoms and findings as well as psychological and sleep problems. Patients with HF have poor sleep quality and this condition is associated with poor quality of life and health status. In a study comparing the sleep characteristics of patients with and without HF, it was found that patients with HF had lower sleep quality and more daytime sleepiness [15]. In another study, 31.3 % of HF patients were reported to show symptoms of chronic insomnia and paroxysmal nocturnal dyspnea, and NYHA classification status was associated with insomnia [16]. In a study conducted to determine the relationship between the course of sleep problems and re-hospitalization in HF patients for one year, it was determined that one-third of HF patients had sleep problems one year after discharge from the hospital. In addition, it was observed that the risk of re-hospitalization of patients who continued to have sleep problems had twice as high. In the studies, it has been reported that sleep problems in patients with HF are related to the disease and treatment, and apart from these, factors such as demographic characteristics, advanced age, gender, comorbid conditions, sedentary life, and the emotional state contribute to sleep disorders [5-7]. Also, changes in sleep patterns seen in patients with HF can negatively affect the prognosis of the disease [17].

In a study by Lee et al., HF patients with PSQI > 5 (HFrEF and HFpEF) were defined as poor sleepers and followed up for one year. 63 % of these patients reported poor sleep quality. It was found that those with poor sleepers had 2.5 times less cardiac event-free survival (95% CI, 1.164 - 5.556) than good sleepers [18]. In a study by Türoff et al., HF patients with EF 45% and below NYHA- FC Class II and above were examined. The presence of whether a breathing disorder during sleep was evaluated by polysomnography. Less restorative sleep may

cause changes in sympathovagal balance and impairment in the reset of important reflexes. This may contribute to poor cardiovascular outcomes in patients with HFrEF [19]. Wang TJ et al., investigated factors affecting sleep quality in patients with HF. The mean PSQI of these patients was 10.78 ± 4.78 and 81 % of the patients had low sleep quality. The most common cause of sleep interruption was urination. Gender, perceived health, depressive mood, and number of comorbidities were seen as factors related to sleep quality [20]. In our study, the total PSQI score of baseline was higher than after treatment. Also, patients with poor sleep quality after treatment were less than baseline.

In the AWAKE-HF study, sacubitril/valsartan and enalapril were compared. In this study, physical activity and sleep were evaluated using actigraphy, which a wearable biosensor. Actigraphy is a non-invasive method that can be evaluated objectively and accurately for 24 hours. No difference in activity or sleep was observed between sacubitril/valsartan and enalapril. However, with sacubitril/valsartan therapy for 16 weeks, it was seen with a statistically significant improvement in health-related quality of life compared to onset [21]. In the PROVE-HF study, significant improvements were observed in left ventricular ejection fraction (LVEF) after ARNI treatment. At 12 months, the LVEF median increased from 28.2 % to 37.8 % [difference, 9.4% (95% CI, 8.8 % - 9.9 %)]. A significant 5.2 % increase in LVEF was also seen as early as 6 months (5.2 %, 95 % CI, 4.8 % to 5.6 %). An absolute LVEF increase of over 13 % was observed in 25 % of patients. Overall, the results of the PROVE-HF study show significant improvements in cardiac structure and function measurements at six months and one year in patients with HFrEF [22]. In our study, there was a significant increase in LVEF after ARNI treatment compared to baseline [30.0 (25.0 - 39.0) vs 26.5 (20.0 - 34.7), $p < 0.001$]. LVEF increased 13.2 % after treatment. In addition, a significant reduction was observed in LV diastolic and systolic diameters after treatment.

In our study, symptomatic hypotension was observed in only three patients during ARNI treatment. These patients who were given the form 49/51 mg were switched to a lower dose (24/26). Two patients who could not tolerate drugs were stopped treatment. In other patients, there were no problems that would stop treatment. In our study, the frequency of responding to the question " I can not breathe comfortably" in item d of the 5th question of the PSQI index decreased after the treatment compared to the baseline. We can comment on this as a decrease in the frequency of paroxysmal nocturnal dyspnea. As a result of our study, ARNI treatment reduces the total PSQI scores in patients with HFrEF. Thus, we can say that the patients had a better quality of sleep after the ARNI treatment.

CONCLUSION

Sleep problems in chronic HF patients are one of the unfavorable clinical conditions. In conclusion, sleep quality increases with ARNI treatment in patients with HFrEF. Patients can sleep more comfortably with this treatment.

Limitations

Although our study is a prospective study, it has some limitations. The first and most important of these is the small study population. Studies with more patients can give more consistent results regarding the sleep quality of ARNI in the treatment of HF. Second, this drug is still not included in the refund coverage of the Ministry of Health in our country. This expensive drug was only can give for a few months in most of the study population. This may be perceived as a tendency to select patients in the study population. Third, due to the illiteracy of some patients, the assistance of the relatives of the sleep questionnaire was conducted by asking questions to the patient. It can not be ruled out that this issue can create bias. Fourth, we only did a sleep assessment with PSQI. Studies with more quantitative methods such as polysomnography or actigraphy may give a better idea to clinicians.

Author contribution

FI originated the idea of the research. FI designed the study. BA, MO, and ET collected data. FI and BA analyzed the data. FI wrote the paper. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Ethical Committee (Protocol No: 319, 04/07/2019).

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

The Potential Use of Elastic Tissue Autofluorescence in Formalin-fixed Paraffin-embedded Skin Biopsies

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ABSTRACT

Autofluorescence (AF) or naïve-florescence is the natural emission of light by biomolecules. During florescence microscope examination, we realized that elastic tissue is brighter or more autoflourescent than collagen and other biomolecules/cells in the skin. Consequently, we decided to review elastic tissue-related pathologies under a florescence microscope and to report the possible benefits of this technique from selected cases from the paraffin-block archive, by using the protease digestion immunofluorescence method. Selected and clinic-pathologically confirmed 3 elastofibroma dorsi, 3 pseudoxanthoma elasticum, 3 anetoderma, 3 arteriovenous malformations, 3 temporal arteritis, 3 scar tissue and 3 highly solar-damaged samples of skin from 2014-2019 were retrieved. Under the fluorescent microscope, coarse, thick and globularly-fragmented elastic fibers of elastofibroma dorsi, shortened, irregular and convoluted elastic fibers of pseudoxanthoma elasticum, internal elastic membranes of arteries and their integrity was visualized. None of the anetoderma cases had any signal representing elastic tissue. It was shown that elastic tissue can be observed easily under fluorescence microscope in the case of FFPE tissues. The resulting autofluorescence can be useful in recognizing elastic tissue-related pathologies, and it may be used as an ancillary or an alternative method to routine histochemical techniques.

Keywords: Autofluorescence, elastic, elastic membrane, skin

INTRODUCTION

Direct immunofluorescence involving frozen sections is the gold standard method to determine immune deposits in skin and renal biopsies. Antigen retrieval with proteinase on formalin-fixed and paraffin-embedded tissue has been used in renal pathology as a salvage technique when no glomeruli or frozen sections are available [1,2]. Recently, the value of direct immunofluorescence on proteinase-digested formalin fixed paraffin-embedded skin biopsies was investigated, and it was found out to be a less sensitive but still valuable technique for the identification of immune deposits on skin [3]. In addition, H&E stained sections have been evaluated under a florescent microscope in the case of alopecia, melanoma and fibrous proliferations [4-8].

Autofluorescence (AF) or naïve-florescence is the natural emission of light by biomolecules. After the invention of florescent optical technology and AF spectroscopy, AF principles have been used as a tool, not only in medical practice [9], but also in a variety of research fields [10-13].

Under a fluorescent microscope, AF is usually a redundant situation due to a reduction in immunofluorescence evaluation quality since it generates confounding signals that hamper or mimic a florescent dye. Different methodologies have been used to reduce such signals and to increase the quality of both immunofluorescence and florescent in-situ hybridization evaluation [14-16]. Autofluorescent biomolecules (fluorophores)

that can be seen under a florescence microscope are consist of elastic, collagen and sweat gland secretions.

During a routine florescence microscope examination, we realized that elastic tissue is far brighter or more autoflourescent than collagen and other biomolecules/cells in the skin. That is to say elastic tissue can easily be identified from background tissues in that it offers a sharp contrast. Additionally, the elastic tissue contrast facilitates the observation of elastic fiber microanatomy. On this basis we decided to review elastic fiber-related pathologies under a florescence microscope and to report the possible benefits of this technique from selected cases from our institution's paraffin block archive, by using the protease digestion immunofluorescence method.

MATERIALS AND METHODS

The study has been performed according to the Declaration of Helsinki. 3 elastofibroma dorsi, 3 pseudoxanthoma elasticum, 3 anetoderma cases which were selected and clinic-pathologically confirmed from the period 2014-2019 were retrieved from the files of our department. In addition, 3 arteriovenous malformations and 3 temporal arteritis cases were included to present the existence and integrity of the elastic membrane respectively, with the help of the elastin's naïve florescence. 3 scar tissue and 3 highly solar-damaged samples of skin were also included.

The proteinase digestion protocol applicable for formalin-fixed paraffin embedded tissues was carried out as it has been previously [3]. 5µ-thick sections were cut from representative blocks and placed on charged slides. Following deparaffinization and rehydration, the slides were incubated in phosphate buffer saline (PBS) for 10 minutes. Antigen was retrieved by protease induction at 37°C with a 0.05% proteinase (Sigma cat # P8038) in PBS for 5 minutes. Tissue sections were incubated for 45 minutes in the dark with FITC (fluorescein isothiocyanate) - conjugated IgG (Dako; dilution,1:20; cat. no. #F0315) and a C3 (Dako; dilution,1:200; cat. no. #F0201) antibody. The sections were then rinsed twice in PBS. The stained slides were cover-slipped with a mounting medium (Dako; cat. #S3023). The slides were evaluated under a florescence microscope.

RESULTS

Similar results were obtained with both a FITC-conjugated IgG, and C3 antibodies. None of the cases had a background collagen autofluorescence signal that would hamper elastic fiber morphology.

Elastofibroma Dorsi: Coarse, thick and globularly-fragmented elastic fibers were easily seen in all cases. At a higher power, serrated edges were demonstrated. No background collagen florescence that could hamper elastic evaluation was identified (Figure 1).

Pseudoxanthoma Elasticum: Under a florescence microscope, all 3 cases had granular, shortened, irregular and convoluted elastic fibers, haphazardly oriented at the mid-dermis (Figure 2).

Anetoderma: All the selected cases were clinically and pathologically correlated with advanced stage anetoderma cases. On hematoxylin and eosin stained slides, each case showed abundant sclerodermoid collagen. Staining was repeated 3 times, but all cases were devoid of an elastic fiber autofluorescence signal (Figure 3).

Temporal Arteritis: Luminal-oriented sections demonstrate a linear autofluorescence of the internal elastic membrane. Discontinuity and fragmentation could easily be identified (Figure 4a-d).

Arteriovenous Malformation

Arteries can be distinguished from surrounding veins in the lesion by noting the autofluorescence of the elastic membrane (Figure 4e-h).

Equivocal and variable results are obtained from the scarred and solar-damaged skin tissues. Both solar elastosis and scarring can hinder or aggravate the autofluorescence signal.

DISCUSSION

Autofluorescence (AF) or naïve-florescence is a natural emission of light by biomolecules. After the invention of florescent optical technology, AF began to be used in research and in the medical field in various ways. For instance, with regard to the determination of dysplastic or cancerous epithelium, AF characteristics were proposed as a

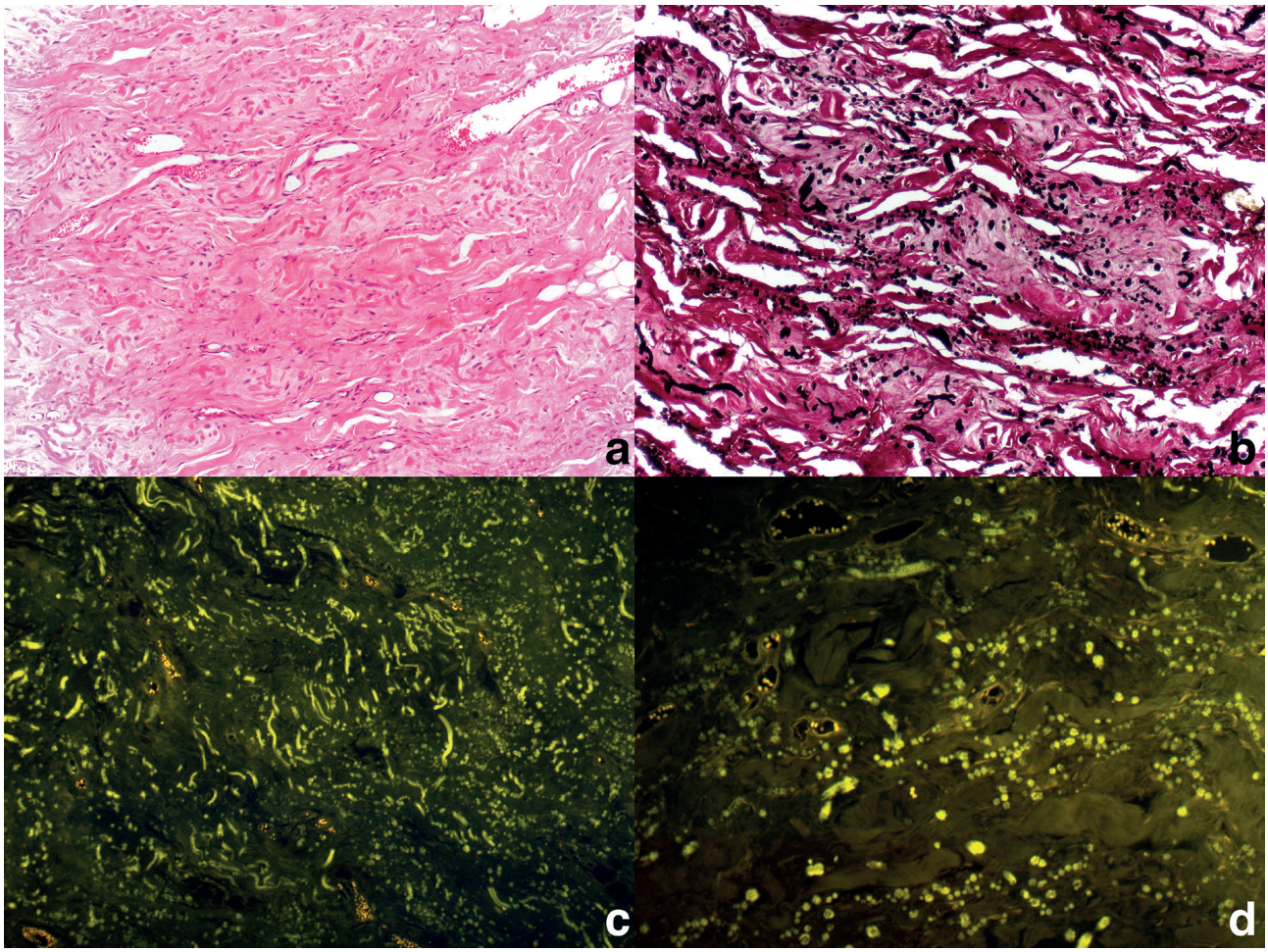

Figure 1. Elastofibroma dorsi a: Thick, globular, elastic fiber fragments are scattered throughout the collagenous stroma (100x) b: Elastica Van Giesson stain (100x) c-d: The autofluorescence of thick, globular elastic fiber fragments can be seen under fluorescence microscope with a minimal collagenous background signal (100x, 200x).

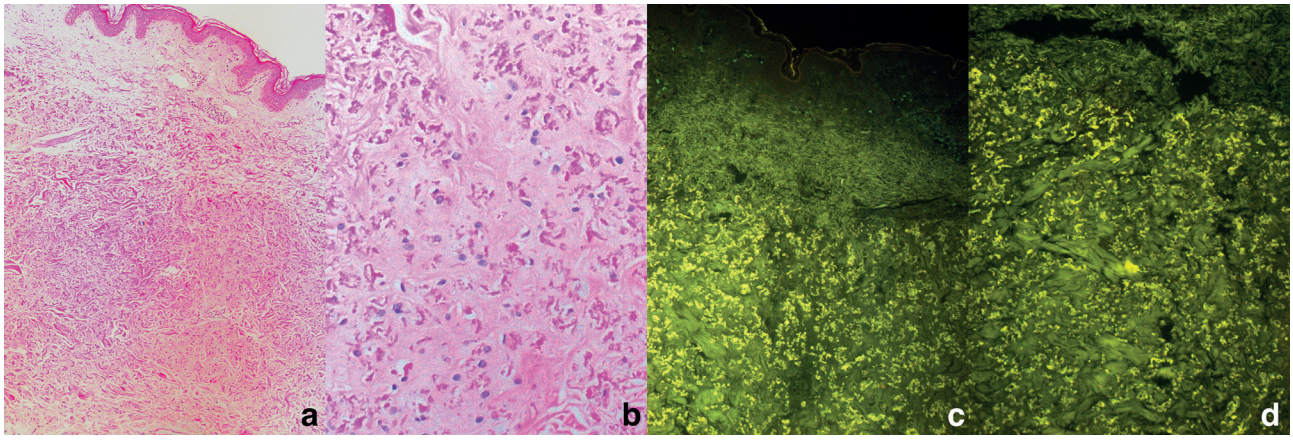

Figure 2. Pseudoxantoma elasticum a: Short, curled and irregular elastic fibers in the reticular dermis. Papillary dermis is spared (100x). b: İrregular fragmentation of the elastic fibers (200x) c-d: Autofluorescence of haphazardly oriented curled elastic fibers in the reticular dermis (100x).

tool for detecting the biopsy site or with regard to deciding on the most appropriate form of clinical management (10-12). It has also been considered for identifying the lung tumor histologic type based on the color textures of AF bronchoscopic images (13). Regarding the skin, AF has been suggested as a useful parameter for detecting skin cancer recurrence [14] and for increasing the reproducibility of the patch test [15]. Moreover, autofluorescence principles have been used in-vivo multiphoton microscopy to provide information with regard to age-related delays in healing, an

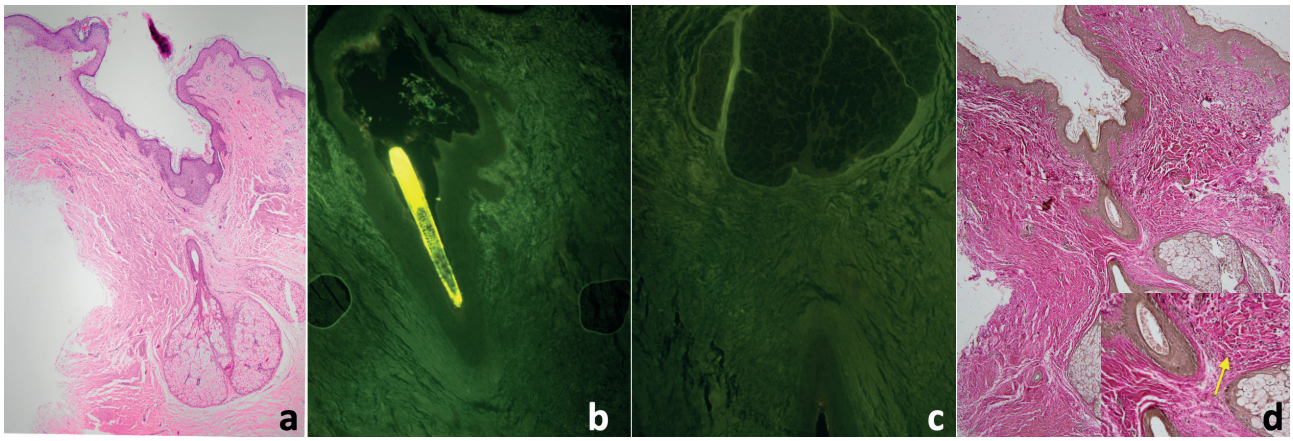

Figure 3. Anetoderma a: Established lesion has increased collagenous activity (40x) b-c: No elastic tissue autofluorescence is identified under fluorescent microscope (100x) d: Elastica Van Giesson stain, elastic fibers are diminished (40x). Residual focal and fragmented black elastic fibers are inner positive control of the stain (inlet-200x).

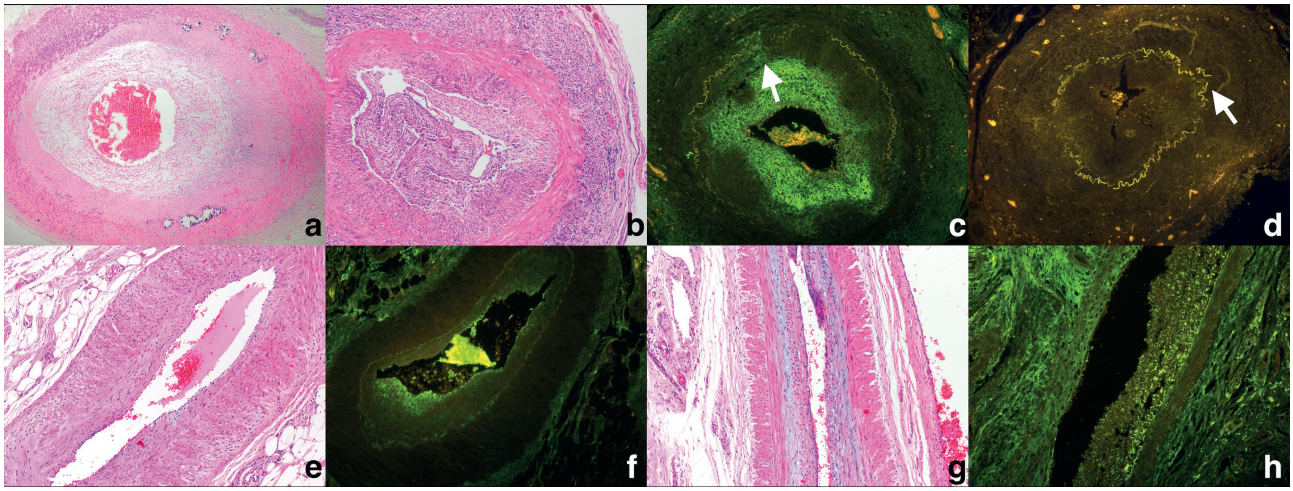

Figure 4. a-b: Clinical-pathological correlated giant cell (temporal) arteritis cases, luminal oriented sections (100x). c-d: Autofluorescence of the elastic membrane helps to examine the integrity or fragmentation of the elastic lamina. The white arrow indicates fragmented lamina (100x). e-f: Luminal orientation of a vessel from arteriovenous malformation case. Autofluorescence of the elastic membrane helps to identify arterial component (100x). g-h: Venous component of arteriovenous malformation, devoid of arterial elastic membrane autofluorescence (100x).

aspect which can be useful for future patient-specific wound care [20].

AF could be redundant since it results in a confounding signal which restricts effective immunofluorescence evaluation. This is especially true in the case of myeloproliferative disease resulting from due to the high level of collagen and elastic production. Hence, the AF characteristics of the elastic tissue under a fluorescence microscope are very well-known.

It is known to be used in nephropathology practice when a frozen section is not available [1,2]. Recently, this technique has been applied to formalin-fixed paraffin-embedded skin tissues [3]. Although in this research the aim was not to detect immune accumulation, the protease digestion protocol was preferred. This is because the authors think that protease digestion will contribute to the brightness of elastic tissue in FFPE tissues. Moreover, an antibody was not needed for generating an AF signal. Because FITC (fluorescein isothiocyanate)-conjugated IgG and C3 antibody consumables were available and ready for use, conjugated antibodies were applied to the FFPE tissues. FITC (fluorescein isothiocanate) or DAPI (4′,6-diamidino-2-phenylindole) could be suitable for detecting elastic AF signals. Consequently, it is expected that both antibodies will give the same results.

"Is it an artery or a vein?" Under a light microscope the answer to this question is sometimes difficult, but if elastic membrane is detected, the answer

has already been given, in that the vessel is most probably an artery. Routine histomorphology practice involving the differential diagnosis of malformative vascular anomalies is challenging. Differential diagnoses include arteriovenous malformation and venous malformation. This distinction can be easily made by identifying the elastic membrane involved. Consequently, this particular question can be answered with the use of a florescence microscope with the help of the elastic membrane's AF characteristics (Figure 4e-h). In the literature we can also find evidence of autoflorescence technology's success in terms of identifying vessel type in dorsal skin, the cerebral cortex, and in large vessels in the abdominal cavity [21]. Moreover, it is clear that the integrity of elastic lamina can easily be recognized with the aid of a florescence microscope. Thus, we decided to retrieve the giant cell arterititis from our institution's archive and stain it with FITC (fluorescein isothiocyanate) - conjugated IgG and C3 antibodies. The diagnostic morphologic clue of giant cell arteritis - a fragmentation of the internal elastic membrane - can readily be identified under a florescence microscope (Figure 4a-d).

Elastic tissue anomalies in the skin, such as pseudoxanthoma elasticum and anetoderma, can also be evaluated under routine florescence microscope examination. In the case of pseudoxanthoma, the elastic tissue microanatomy can easily be visualized. No elastic tissue signal was recognized in anetoderma cases (Figures 2 and 3) although it was repeated 3 times. When the issue is early anetoderma biopsy, the comparison of lesional skin with intact skin by quantifying the AF signal, might potentially be the subject of further research. It may be the case that in the future, diagnosis might be made by comparing AF signals obtained from an anetoderma lesion and non-lesional skin. Perhaps, as a result of these studies, a threshold value might be determined for a precise distinction to be made.

Equivocal and variable results are obtained in the case of scarred and solar-damaged skin tissue. The AF signal is aggravated in some cases, whereas in some cases it is attenuated. This may be due to the variability of the presence and amount of intact elastic fibers in tissues associated with the aforementioned morphology.

In routine pathology practice, elastic fiber stains such as elastica Van Giesson or Verhoeff elastic stains have been used to detect elastic fibers. However, with regard to these stains it can be technically difficult to achieve the required standard, and microscopic control is necessary between staining steps. In contrast, immunofluorescence microscopy is a technique that requires some microscope use experience. However, the fact that the AF technique is standard and does not require microscopic control is a situation that facilitates its applicability. Which type of elastic fiber is more autofluorescent, or which is superior to the routine elastic histochemistry, are questions that are waiting to be answered. Specificity and sensitivity results can be obtained by comparing the protease-mediated results with histochemical studies. Measuring and comparing quantified AF and the histochemistry signals of large series can give such a result.

Autofluorescence occur in H&E sections on IF microscope. Dr. Elston and his team searched for elastic paterns on H&E stained slides of alopecia, fibrous proliferations and melanocytic lesions under a florescent microscope to get rapid assessment of elastic pattern [4-8]. However, in our experience, elastic fibers appear more contrast and brighter than the surrounding tissues in the protease digestion method.

In summary, it was shown that elastic tissue can be observed easily under a fluorescence microscope in formalin-fixed paraffin-embedded tissues. The resulting autofluorescence can be useful in recognizing elastic tissue-related pathologies. Moreover, this technique can be used as an ancillary or an alternative method to routine histochemical techniques. In addition, we believe that the quantification of elastic tissue autofluorescence under a fluorescence microscope is a very promising research field, and a method for use in the case of the abovementioned elastic fiber-related pathologies.

Author contribution

Study conception and design: DAO; data collection: DAO and KS; analysis and interpretation of results: DAO and KS; draft manuscript preparation: DAO. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Not applicable, anonymity of the patients' and their confidentiallity was preserved.

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Comparison of Clinical Profiles, Angiographic Features and Outcomes of Young and Elderly Patients with ST-Segment Elevation Myocardial Infarction

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ABSTRACT

Objective: It is to reveal the differences between clinical, angiographic, in-hospital and one-year follow-up results between young and elderly patients with acute ST-segment elevation myocardial infarction.

Materials and Methods: This study was designed retrospectively in two centers. 2891 patients were screened; 260 of which were young. 260 elderly patients were randomly selected among the patients and the differences between both groups were evaluated.

Results: The median age of the young patients was 37 (34-39) years and the median age of the elderly patients was 65 (56-73) years, and male gender was dominant in both groups. Young patients were more likely to be admitted with Killip class 1, while older patients were more likely to be admitted with Killip class 2 (P=0.002). Single-vessel disease was more common in young patients (81.0% vs. 46.3%; P<0.001), while multi-vessel disease was more common in the elderly patients (19.0% vs. 53.7%; P<0.001). In one-year follow-up, all-cause hospitalization was lower in younger patients, but there was no significant difference in mortality between elderly and young patients.

Conclusion: Young patients presenting with ST-segment elevation myocardial infarction were more frequent smokers, obese and dyslipidemic and although in-hospital outcomes were better than the elderly, one-year mortality was similar to those of the elderly.

Keywords: Myocardial infarction, young patients with MI, elderly patients, outcome

INTRODUCTION

ST-segment elevation myocardial infarction (STEMI) is one of the most common causes of emergency room admission and cardiovascular death and therefore currently poses a major burden on healthcare worldwide [1,2]. Although the incidence of acute coronary syndrome has decreased in the elderly population in recent years, unfortunately, there is no decrease in the incidence of STEMI in young people [3]. In some studies, although the short-term prognosis of acute STEMI is favorable in young patients, it has been reported that the long-term prognosis is unfavorable [4]. STEMI, with an increasing incidence in young adults, is an important problem for both the patient and the treating physician [5,6]. In recent studies, it was stated that the prevalence of acute STEMI increased significantly in young people. Therefore, recent studies have focused on the clinical profile, risk factors, treatment and long-term consequences of premature myocardial infarction (MI) in young people [7]. While it is important to have MI at an early age, there is not enough data on the etiology and long-term prognosis of this disease, since the most productive life years of the society are at risk

due to this disease. In this study, it was aimed to compare the clinical profiles, risk factors, in-hospital and one-year clinical outcomes of young and old patients with STEMI.

MATERIALS AND METHODS

Study population

This study was carried out retrospectively at two centers between June 2015 and June 2020. The study included 260 patients younger than 45 years of age who applied to the emergency department with the diagnosis of acute STEMI and were hospitalized in the coronary intensive care unit who underwent reperfusion therapy. 260 patients older than 45 years who were admitted to the hospital during the study period were randomly selected. The clinical profiles, risk factors, angiographic features, in-hospital and one-year clinical outcome of these patient groups were compared. Demographic information of the patients, age, gender, coronary artery disease (CAD) risk factors, cardiovascular disease histories, laboratory results were obtained from the records in the electronic system of the hospitals. The family histories and one-year results of the patients were learned by telephone call method.

Definitions

In this study, patients were divided into two groups, according to the current European Society of Cardiology guidelines, patients younger than 45 years were defined as "young" and patients older than 45 years were defined as elderly [8]. STEMI was defined according to the 4th universal MI guideline [9]. The type and localization of myocardial infarction were determined according to electrocardiographic (ECG) findings. The patients were divided into two groups according to their body mass index (BMI) (<25 and >25 kg/m2). Patients with a body mass index >25 kg/m2 were defined as obese. Dyslipidemia was defined as serum total cholesterol (TC) ≥200 mg/dl; triglyceride (TG) >150 mg/dl; low-density lipoprotein (LDL) >130 mg/dl; high-density lipoprotein (HDL) <50 mg/dl in men and <40 mg/dl in women; and/or those receiving lipid-lowering therapy [10]. The term re-infarction was defined as the ischemic symptoms lasting 20 minutes or longer and at least one of the following: recurrence of ≥0.1 mV ST-segment elevation in at least two contiguous leads on the ECG, or the appearance of new pathognomonic Q waves, and a 20% or greater increase in cardiac troponin [11]. TIMI thrombus scale was used to evaluate thrombus burden [12]. Then, the TIMI thrombus score was divided into two classes as large thrombus grade (4 and 5) and small thrombus grade (1-3 grade)[13]. Castelli's risk index 1 and 2 (CRI-1 and CRI-2) were defined as TC/HDL-c and LDLc/HDL-c ratios. In-hospital outcomes included left ventricular ejection fraction (LVEF) before discharge, reinfarction, cardiogenic shock, stroke, major bleeding, non-major bleeding, blood transfusion and all-cause mortality. One-year clinical outcome was defined as hospitalization for any cause, MI, coronary angiography, cardiovascular death and all-cause death. Patients with the following conditions were excluded from the study: Congenital heart disease, cardiomyopathy, myocarditis, MI due to aortic dissection, Takayasu arteritis or vascular dysplasia, those who did not undergo coronary angiography who had an acute MI during pregnancy and in-hospital. The study was approved by the local ethics committee.

Statistical Analysis

Continuous data are given as median (Q1 - Q3). Categorical data are given as a percentage (%). Shapiro Wilk's test was used to investigate the suitability of the data for normal distribution. The Mann-Whitney U test was used for the cases with two groups in the comparison of those which did not conform to the normal distribution. Pearson Exact Chi-Square analyzes were used in the analysis of the created cross tables. IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) program was used in the analysis. A value of $p<0.05$ was accepted as a criterion for statistical significance.

RESULTS

Demographic findings and risk factors

A total of 2891 acute STEMI patients, 260 (9%) of which were young (≤45 years), were screened for inclusion in the study. The median age of the young patients was 37 (34-39) years and the median age of the elderly patients was 65 (56-73) years. While the male gender was dominant in both groups,

there was no statistically significant difference in both groups (P=0.529). When evaluated in terms of CAD risk factors, diabetes mellitus (DM) (35.5% vs. 13.8%; P<0.001), hypertension (HT) (52.5% vs. 15.4%; P<0.001), CAD history (28.6% vs. 10.0%; P<0.001), chronic renal failure (CRF) (5.4% vs. 0.4%; P<0.001) were significantly more common in the elderly than in the young patients. Smoking (65.1% vs. 43.4%; P<0.001), dyslipidemia (34.5% vs. 16.6%; P<0.001), family history (23.6% vs. 5.0%; P<0.001) and BMI>25 kg/m2 (25.0% vs. 20.0%; P=0.035) were more common in young people. Considering the duration of admission to the hospital and symptoms, younger patients were more likely to be admitted with Killip class 1, while the elderly were more likely to be admitted with Killip class 2 (P=0.002). The median time from symptom onset to admission to the emergency department was detected longer in the elderly [60(45-120) vs. 45(35-90); P<0.001]. When evaluated in terms of the type of MI, while young people were more likely to present with anterior MI, the elderly were more likely to present with inferior MI [55.8% vs. 36.2%), (52.3% vs. 28.5%); P<0.001 for both] (Table 1).

Laboratory findings

When evaluated in terms of laboratory parameters, hemoglobin (Hgb) (15.4±1.5 vs. 14.4±1.89; P<0.001), TC (209.4±55.2 vs. 189.3±43,8; P<0.001), LDL-C (132.3±45.0 vs. 125.4±36.4; P=0.049) and TG (187.4±123.4 vs. 148.2±86.1) values were found to be higher in young patients, while HDL-C (40.9±9.6 vs. 37.4±7.1; P<0.001) was lower in young patients than in the elderly (Table 2).

Angiographic findings

In terms of coronary angiographic findings, the duration of fluoroscopy was shorter in the young compared to the elderly (25.06±11 vs. 33.9±12; P<0.001). As a result of coronary angiography performed after STEMI, normal coronary arteries (5.1% vs. 1.2%; P<0.005) and single vessel disease (81.0% vs. 46.3%; P<0.001) was more common in the young, whereas multivessel disease was more common (53.7% vs. 19.0%; P<0.001) in the elderly. When the thrombus burden was examined, there was no significant difference between both groups in terms of small thrombi, while large thrombi (50.4% vs. 33.1%; P<0.001) were significantly more common in the young than in the elderly. Angiographic findings are summarized in table 3 in detail.

Comparison of findings of in-hospital and one-year clinical outcomes

When evaluated in terms of in-hospital clinical outcomes, length of stay in intensive care unit (2(2-3) vs. 1(1-1) days; P=0.026), major bleeding (3.1% vs. 0.4%; P= 0.018), re-infarction (5.4% vs. 2.3%), cardiogenic shock (12.7% vs. 3.1%; P<0.001), LVEF before discharge (54.56±8 .6 vs. 46.36±10.9%, and in-hospital mortality (8.8% vs. 1.9%; P<0.001) differed significantly between both groups.

In 1-year clinical outcomes, all-cause hospitalizations (43.0% vs. 25.7%; P=0.001) and hospitalizations due to heart failure (HF) (12.7% vs. 5.1%; P=0.005) were more common in the elderly, but there was no difference between both groups in terms of MI, revascularization, stroke, all-cause death and cardiovascular death. In-hospital and one-year outcomes are detailed in Table 4.

DISCUSSION

In this retrospective study performed in two centers evaluating 520 patients including 260 young and 260 elderly, patients aged ≤45 years constituted 9% of the total study population and male gender was more dominant (83.8%). More than half (65.1%) of the young patients were smokers, one-third (34.5%) had dyslipidemia and 23.1% had a history of CAD in their first-degree relatives, and also 25% of the youth were obese. Low density lipoprotein cholesterol in the blood values taken in the first 24 hours were found to be higher in young patients, while HT, DM and kidney failure were higher in the elderly. Castelli risk index (CRI) 1 and 2 were found to be higher in young patients than in the elderly. Although the in-hospital outcomes of patients aged ≤45 were better, there was no difference in mortality in the one-year follow-up compared to patients aged >45 years. Although there is not enough data on the incidence of acute MI in young people, it has been reported to be between 4-10%. In our study, the incidence of MI was found to be 9%, which is consistent with other studies, although it is higher than the Norwegian registry study [14]. Because the definition of acute MI has changed a few times over the past decade and various studies have used different inclusion criteria, it is difficult to compare incidence rates through different time periods and in different populations [15,16]. The clinical presentation of CAD in patients younger than 45

Table 1. Comparison of baseline characteristics and risk factors of the study population.

Variables	≤45 years, STEMI n=260	>45 years, STEMI n=260	P
Median (IQR), age, years	37(34-39)	65(56-73)	<0.001
Male, n (%)	218(83.8%)	195(75%)	0.529
CAD Risk factors			
DM, n (%)	36(13.8%)	92(35.5%)	<0.001
HT, n (%)	40(15.4%)	136(52.5%)	<0.001
Chronic renal failure, n (%)	1(0.4%)	14(5.4%)	<0.001
CAD history, n (%)	26(10.0%)	74(28.6%)	<0.001
Smoking, n (%)	168(65.1%)	112(43.4%)	<0.001
Dyslipidemia, n (%)	82(34.5%)	43(16.6%)	<0.001
Family history, n (%)	61(23.6%)	13(5.0%)	<0.001
BMI >25 kg/m^2, n (%)	65(25%)	52(20%)	0.035
Hospital admission symptom			
Typical angina, n (%)	248(95.4%)	233(90.0%)	<0.001
Dyspnea, n (%)	44 (16.9%)	75(29%)	<0.001
Syncope, n (%)	0(0.0%)	15(5.8%)	0.073
Prehospital cardiac arrest, n (%)	14 (5.4%)	15 (5.8%)	0.500
Killip classification			0.002
• Class I	223 (85.5%)	188 (72.3%)	
• Class II	23 (8.8%)	37 (14.2%)	
• Class III	8 (3.1%)	20(7.7%)	
• Class IV	6(2.3%)	15(5.8%)	
Time from symptom onset - to ED (Q1-Q3)[a]	45(35-90)	60(45-120)	<0.001
Type of myocardial infarction			
Anterior myocardial infarction, n (%)	145 (55.8%)	94 (36.2%)	<0.001
Anterolateral myocardial infarction, n (%)	17 (6.5%)	5 (1.9%)	0.007
Inferior myocardial infarction, n (%)	74 (28.5%)	136 (52.3%)	<0.001
Posterior myocardial infarction, n (%)	15 (5.8%)	11(4.2%)	0.273
Other type of MI	9 (3.5%)	14 (5.4%)	0.197

[a] These times were calculated on 426 patients due to the missing data of some patients.

Abbreviations: BMI: body mass index, CAD: coronary artery disease, ED: emergency department, DM: diabetes mellitus, HT: hypertension, MI: myocardial infarction.

Table 2. Comparison of laboratory values between the two groups.

Variables	≤45 years, STEMI n=260	>45 years, STEMI n=260	P
Hemoglobin, g/dL, ±SD	15.4±1.5	14.4±1.89	<0.001
WBC, g/dl, (Q1-Q2)	11.5(9.0-14.8)	11.4(9.31-14.8)	0.832
Neutrophil, 10^3 /µL, (Q1-Q2)	7.13(5.2-10.3)	7.7(5.63-10.71)	0.150
PLT, (Q1-Q2)	254(215.5-314.2)	237(193.0-289.0)	0.005
Glucose, mg/dL, (Q1-Q2)	112(98-142)	150(120.5-216.7)	<0.001
Creatinine, mg/dL, (Q1-Q2)	0.86(0.76-1.03)	0.98(0.82-1.19)	<0.001
Na, ±SD	137±3.1	137±3.44	0.701
K, ±SD	4.2±0.4	4.38±0.63	0.010
Total cholesterol, g/L, ±SD	209.4±55.2	189.3±43.8	<0.001
LDL cholesterol, g/L, ±SD	132.3±45.0	125.4±36.4	0.049
HDL cholesterol, g/L, ±SD	37.4±7.1	40.9±9.6	<0.001
TG, g/L, ±SD	187.4±123.4	148.2±86.1	<0001
Castelli 1, ±SD	5.78±2.1	4.8±1.3	<0.001
Castelli 2, ±SD	3.63±1.65	3.18±1.05	0.001

Abbreviations: HDL: high density lipoprotein, K: potassium, LDL: low density lipoprotein, Na: sodium, PLT: platelets, TG: triglyceride, WBC: white blood cell.

Table 3. Comparison of angiographic findings and in-hospital medications of the young and old patients.

Variables	≤45 years, STEMI n=260	>45 years, STEMI n=260	P
Radial access, n (%)	11(4.2%)	3(1.2%)	0.020
Femoral access, n (%)	245(94.6%)	256(98.8%)	0.010
Fluoroscopy time, ±SD	25.06±11	33.9±12	<0.001
Fibrinolytic, n (%)	1(0.4%)	1(0.4%)	-
Primary coronary intervention, n (%)	237 (97.1%)	243(98.3%)	0.590
DES, n (%)	211(87.2%)	213(82.9%)	0.054
BMS, n (%)	12(5.0%)	6(2.3%)	0.019
CABG, n (%)	14(5.4%)	37(14.3%)	0.001
Number of involved vessels			
Normal coronary, n (%)	13 (5.1%)	3(1.2%)	0.011
Single vessel, n (%)	200(81.0%)	118(46.3%)	<0.001
More than one vessel, n (%)	47(19.0%)	137(53.7%)	<0.001
Culprit coronary artery			
LMCA, n (%)	6(2.3%)	4(1.6%)	0.385
LAD, n (%)	151(58.1%)	97(37.9%)	<0.001
CX, n (%)	31(11.9%)	38(14.8%)	0.199
RCA, n (%)	55(21.2%)	104(40.6%)	<0.001
Other coronary arteries, n (%)	6 (2.3%)	10 (3.9%)	0.214
Thrombus burden			
Small thrombus, n (%)	82(31.5%)	72(27.7%)	0.194
Large thrombus, n (%)	131(50.4%)	86(33.1%)	<0.001
Thrombus aspiration, n (%)	20 (7.8%)	3(1.2%)	0.001
Before the procedure TIMI flow 0-1	243(93.5%)	225 (87.9%)	0.021
After the procedure TIMI flow 3	252(96.9%)	232(89.9%)	0.001
Medications			
Acetylsalicylic acid, n (%)	252(99.6%)	259(100%)	0.494
Clopidogrel / prasugrel / ticagrelor, n (%)	252(99.6%)	247(96.6%)	0.386
LMWH, n (%)	236(92.6%)	251(97.3%)	0.008
Glycoprotein 2b/3a inhibitors, n (%)	73 (28.6%)	23(8.3%)	<0.001
Betablockers, n (%)	127(50.4%)	158(61.2%)	0.008
Statin, n (%)	246(95.3%)	231(90.6%)	0.026
ACEI/ARB, n (%)	104(40.8%)	144(55.8%)	<0.001

Abbreviations: ACE: angiotensin converting enzyme, ARB: angiotensin receptor blocker, BMS: Bare metal stent, CABG: Coronary bypass graft surgery, DES: Drug eluting stent, IV: Intravenous, PCI: Percutaneous coronary intervention, PTCA: percutaneous coronary angioplasty.

years old may differ from that in older patients. Typical chest pain, which is the first symptom of acute MI, was more common in the young than in the elderly, consistent with previous studies [6,17]. While young people were mostly admitted with Killip class 1, the elderly were admitted more with Killip class 2, 3 or 4. In previous studies, it was stated that elderly patients were more likely to present with HF symptoms due to the prevalence of advanced CAD and low LVEF [18-20].

In young people with acute MI, it is common to have multiple risk factors and the majority of these patients are reported to have at least one traditional cardiovascular risk factor [21,22]. Smoking is the most preventable universal cause of death by leading to the initiation and progression of atherosclerosis [23]. Smoking cessation greatly reduces the risk of CAD and there are striking results reporting that giving up before age 40 reduces the risk of death by 90% [23]. Consistent with previous studies, in our study, the rate of smoking was higher in young people than in the elderly (43.4% versus 65.1%)[7,24,25]. These data explain the importance of smoking cessation over early MI [26]. Low density lipoprotein cholesterol and TG values were found to be higher in young people than in

Table 4. Comparison of in-hospital and one-year clinical outcomes of young and elderly patients.

Variables	≤45 years, STEMI n=260	>45 years, STEMI n=260	P
In-hospital clinical outcomes			
Total hospital stay, days (IQR)	4(3-5)	4(3-6)	0.232
ICU stay, days (IQR)	1(1-1)	2(2-3)	0.026
Major bleeding, n (%)	1(0.4%)	8(3.1%)	0.018
Requiring for blood transfusion, n (%)	1(0.4%)	9(3.5%)	**0.010**
Re-infection, n (%)	6(2.3%)	14(5.4%)	**0.054**
Cardiogenic shock, n (%)	8(3.1%)	33(12.7%)	<0.001
Stroke, n (%)	1(0.4%)	2(0.8%)	0.499
AV block, n (%)	4(1.5%)	16(6.2%)	0.005
VT, n (%)	9(3.5%)	17(6.5%)	0.079
VF, n (%)	23(8.8%)	17(6.5%)	0.205
AF, n (%)	7(2.7%)	17(5.4%)	0.095
LVEF before discharge ±SD[a]	54.5±8.6	46.3±10.9	<0.001
Death, n (%)	5(1.9%)	23(8.8%)	<0.001
One-year clinical outcome			
All-causes hospitalization[b], n (%)	66(25.7%)	95(43.0%)	0.001
Hospitalization due to HF, n (%)	11(5.1%)	33(12.7%)	0.005
Myocardial infarction[c], n (%)	11(4.3%)	12(5.5%)	0.349
Revascularization[c], n (%)	44(17.1%)	44(20.1%)	0.237
Stroke, n (%)	1(0.5%)	3(1.2%)	0.400
Cardiovascular mortality[c], n (%)	4(1.6%)	7(3.2%)	0.189
All-causes death[d], n (%)	1(0.4%)	2(0.9%)	0.444

[a] Since 20 patients did not have LVEF' information, it was calculated over 500 patients.

[b] Since the data of 42 patients could not be reached; analysis was performed on 478 patients.

[c] Since the data of 44 patients could not be reached; analysis was performed on 476 patients.

[d] The data of 3 patients from the young patient group and 39 patients from the elderly patient group could not be reached.

Abbreviations: AV: Atrioventricular, AF: Atrial fibrillation, ICU: Intensive care unit, LVEF: Left ventricular ejection fraction, VF: Ventricular fibrillation, VT: Ventricular tachycardia.

the elderly, while HDL was found to be lower in this study, as well. These results were consistent with previous studies [27,28]. High TG and low HDL levels, which characterize the dyslipidemia aspect of the metabolic syndrome, have a significant role in the development of atherosclerosis and coronary heart disease. In addition, BMI>25 kg/m2 was also significantly higher in the young than in the elderly in this study. All these results show that it is important to carefully examine young patients in terms of metabolic syndrome, which is one of the most important causes of CAD and atherosclerosis [29]. In previous studies, it has been reported that CRI is an important risk index in terms of CAD and cardiovascular outcomes in the elderly and in patients with DM [30,31]. However, there is no information regarding its importance in young people with CAD. Although it has been reported that LDL increases with age in some previous studies [14], it has been shown that LDL value is higher in young people in recent studies. In this study, both CRI 1 and CRI 2 were found to be higher in young people. Of course, the results of our study may not be sufficient to predict future clinical outcomes of CRI alone. Larger prospective studies are required on this subject.

The relationship between a positive family history and early CAD and increased plaque burden is well known. In our study, 1 out of every 5 young patients had a positive family history, and it was significantly higher in younger patients than in patients aged >45 years. Although this rate was reported as 10% in some previous studies, the results of our study were similar to those of most recent studies [23,32].

Consistent with the results of previously reported studies, in our study, normal coronary (5.1%) and single vessel disease (81.0%) were more common in patients aged ≤ 45 years, while multi-vessel disease (53.7%) was dominant in patients aged >45 years [20,23,29,32,33]. In the meanwhile, similar to the results of previous studies, in our study, left

anterior descending artery (LAD) was the most common involved artery (58.1%) in the young, so was the right coronary artery (RCA) (40.6%) in the elderly [20,34]. In addition, the thrombus load was significantly higher in young patients than in the elderly, and therefore more thrombus aspiration was performed in young patients. This result can be attributed to the fact that younger patients with acute MI are more prone to thrombus formation. Consistent with our study, a high thrombus burden was reported in young patients in the study of Shalaby et al. [7].

Under the light of clinical outcomes, the outcomes of our study are similar to previous studies in terms of in-hospital clinical outcomes, but differ in one-year mortality (Table 4). As in this study, in previous studies, young patients with acute MI had a better prognosis than the elderly in terms of length of stay in the intensive care unit, in-hospital cardiogenic shock, HF and mortality. These results can be attributed to the earlier admission of young patients to the hospital, fewer co-morbidities, more frequent single vessel involvement and less frequent severe atherosclerosis [20,35,36]. Previous data suggest that younger patients with MI have a relatively favorable short and long-term prognosis compared with older patients. However, some studies have shown an alarming 15% reduction in survival at 7 years, after 5 years of MI [37-39]. In a recent study, in which the age of 35 was taken into account, it was shown that there was no significant difference in prognosis between both groups in a 4-year follow-up [39]. In our study, in terms of one-year results, while young patients were more advantageous in terms of all-cause hospitalization and hospitalization due to HF, there was no statistically significant difference between elderly and young patients in terms of mortality. In a study published by Fach A et al. in 2019, only a few of 277 young MI patients achieved the risk control goals planned for long-term follow-up after MI. The target level was achieved in 14.8% for body mass index and 27% for LDL level, and the results were shown to be even worse in the follow-up [40]. One of the reasons why the mortality result in our study differs from previous studies can be explained by the progressively worsening of MI prognosis in younger patients over time compared to the past. Another reason may be the small number of patients included in the study and the fact that 43 patients could not be reached during one-year follow-up.

Our study has some limitations. Firstly, it is a retrospective study with most of the patients' data from hospital records. Secondly, the patients were divided into two groups and the control group was randomly selected from the entire patient population as the number of young patients. Third, conditions such as genetic diseases and spontaneous coronary artery dissection, which are among the most important etiologies of acute MI at an early age, could not be evaluated due to the retrospective nature of the study.

CONCLUSION

Young patients presenting with STEMI were more frequent smokers, obese and dyslipidemic. These patients also had greater thrombus burden and a greater prevalence of single vessel disease. In addition, one-year mortality outcomes were similar to those in the elderly although in-hospital outcomes were better in younger patients. These results highlight the importance of smoking cessation, weight loss programs and health education, especially for the population of this age.

Author contribution

Study conception and design: BM; data collection: SM and BM; analysis and interpretation of results: BM; draft manuscript preparation SM and BM. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Eskisehir Osmangazi University ethical committee (2020-438/03/11.2020).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

The Relationship Between ST-Segment Depression in Lead aVR and Coronary Microvascular Function in Acute Inferior Myocardial Infarction

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ABSTRACT

Objective: The aim of this study was to investigate the relationship between ST-segment depression in the aVR lead and coronary microvascular function in acute inferior myocardial infarction undergoing primary percutaneous intervention.

Methods: 287 patients with inferior myocardial infarction confirmed by coronary angiography were divided into two groups with and without ST-segment depression in lead aVR ≥ 0.1 mV on the 12 lead ECG. Electrocardiographic recordings were made for the evaluation of ST-segment resolution before and after primary PCI. Angiographic assessment in the infarct-related artery was performed by using the myocardial blush grade and thrombolysis in myocardial infarction flow.

Results: Overall, 51 of 287 patients had ST-segment depression in lead aVR. The number of patients with RCA-induced infarction was higher in the group with ST-segment depression in lead aVR. RCA involvement was present in 44 patients. Peak troponin was higher in the group with ST-segment depression in lead aVR compare to the other group (P <0.001). The MBG was more impaired, and the STR was less regressed in patients with ST depression in lead aVR (p<0,001). The ejection fraction of patients with ST-segment depression in lead aVR was lower.

Conclusion: We found that ST-segment depression in lead aVR was associated with impaired myocardial perfusion in patients with inferior myocardial infarction.

Keywords: Lead aVR, microvascular function, inferior myocardial infarction, myocardial blush grade

INTRODUCTION

Inferior myocardial infarction (MI) can be seen by the formation of transmural myocardial ischemia as a result of complete occlusion of the right coronary artery (RCA) or left circumflex artery (CX). The first electrocardiogram provides prognostic and clinic information and guides the treatment and follow-up process in case of an emergency.

The importance of lead aVR in determining infarct-related artery (IRA) in patients with inferior MI is uncertain. The lead aVR is often overlooked in traditional clinical practice because it is not adjacent to other electrocardiographic leads. Infarction of this critical area often leads to ST-segment elevation in lead aVR [1,2]. In a recent study, ST-segment depression in lead aVR has been associated with circumflex artery stenosis [3]. The previous studies demonstrated that ST-segment depression (STD) in lead AVR was related to impaired myocardial perfusion [4,5]. Thrombolysis in the flow of myocardial infarction (TIMI) 3 is known as effective recanalization of the epicardial vessel and is a key goal of therapeutic procedures. However, TIMI 3

flow does not always reflect the perfect myocardial reperfusion. Microvascular reperfusion is extremely associated with cardiovascular outcomes. The ST-segment resolution (STR) and myocardial blush grade (MBG) are electrocardiographic and angiographic markers of myocardial perfusion. During coronary angiography, the MBG assesses the degree of washing myocardial blush. A relation has been found between microvascular reperfusion and heart failure, left ventricular dilation, and mortality in several studies [6-8]. Furthermore, MBG during reperfusion has been shown to be a useful parameter in determining the prognosis of patients with acute MI [9,10].

All leads on the ECG, except lead aVR, are used to diagnose the occurrence, severity, and location of acute MI. The lead aVR is often ignored, although it provides useful information. Many studies have shown that even following successful re-canalization of the IRA, some cases do not experience complete cardiac reperfusion [10,11]. Therefore, early and simple tests should be used quickly to determine the high-risk patients. As a result, the goal of this study is to investigate the relationship between STD in lead aVR and coronary microvascular function in patients with inferior MI who had undergone primary percutaneous coronary intervention (PCI).

MATERIALS AND METHODS

Patients

Between January 2018 and December 2019, 298 patients were admitted to the coronary care unit with acute inferior or inferior plus lateral/apical MI who had undergone primary PCI. Patients were divided into two groups: those with STD in lead aVR and those without depression in lead aVR. STR and MBG were used for the evaluation of microvascular perfusion. The groups were classified as impaired reperfusion (STR <70% and MBG: 0-1) and normal reperfusion (STR >70% and MBG: 2-3).

Patients with the following conditions were excluded from the study: 1) a history of non-ST elevation MI, coronary artery bypass surgery, or PCI; 2) chronic kidney failure; 3) evidence of bundle branch block in the ECG; 4) patients with a heart valve prosthesis; 5) patients with insufficient coronary angiographic images for MBG evaluation. A total of 52 patients were excluded from the analysis; thirty-nine patients due to insufficient coronary angiographic images for MBG evaluation, four patients due to a history of coronary artery disease, three patients due to a history of coronary artery bypass surgery, two patients due to a history of heart valve prosthesis, two patients due to chronic kidney failure, and two patients due to left bundle branch block. After exclusions, 287 patients were enrolled.

Clinical, demographic, laboratory parameters, and echocardiography results were recorded for each patient on the first day of admission. Blood samples were collected from all patients on admission and then daily until the patients were discharged. Infarct-related artery, culprit lesion, lesion length, and other angiographic findings were recorded for each patient. The local ethics committee of our institution approved the study.

Electrocardiographic analysis

The ECG was examined for each patient, and the ST-segment was measured 80 ms after the J point at admission and 90 minutes after primary PCI. The diagnosis of inferior MI is based on ischaemic symptoms with a new 1 mm ST-segment elevation in two or more inferior leads (II, III, and aVF).

The STR is defined as the return of the elevated ST segment to the baseline over time. An ECG was obtained for the evaluation of the lead aVR on hospital admission, and STR was evaluated within 90 minutes after the procedure by two independent observers who were blinded to all patients' data. Intra- and inter-observer variability were both low (1% and 2%, respectively). Patients with an STD of at least 1.0 mm in lead aVR were included in the STD group, and an STD ≥2 mm was considered clinically significant. For ST segment resolution, the greatest ST segment elevation in the baseline ECG was used as a reference for the subsequent assessments of ST segment elevation. Microvascular function was considered normal in patients with ST segment resolution> 70%.

Coronary Angiography

The coronary angiography recordings were reviewed by two researchers who were not informed of the ECG findings. Coronary artery stenting (drug-eluting stent) was performed after initial balloon angioplasty. All patients received

300 mg aspirin and a 180 mg loading dose of ticagrelor, as well as intravenous heparin at 60-70 IU/kg i.v. before the procedure and 70-100 IU/kg i.v. during PCI. Using the glycoprotein IIb/IIIa inhibitor (tirofiban) was based on the decision of the operatör. During angiography, MBG was utilized to evaluate the washout of the myocardial blush. (11). The MBG was visually assessed in a catheterization laboratory using a sine film at a rate of 25 frames per second. In this study, coronary angiographic records were sufficient to monitor the contrast filling in the venous phase, and the coronary angiographic records were evaluated using the same images as the IRA. In the evaluation of MBG, a single vision was chosen from multiple orthogonal visions to minimize overlapping of non-infarcted regions. For the circumflex coronary artery, the lateral or right anterior oblique (RAO) pose was used, while the RAO pose was used for the right coronary artery. The TIMI flow grade was evaluated as described [12].

Echocardiography

Within 48 hours after primary PCI, an echocardiographic evaluation was performed according to the American Society of Echocardiography guidelines by two cardiologists who were blind to the study [13].

Statistical Analysis

SPSS version 24.0 was used for the analysis. The Shapiro-Wilks test was utilized to verify the distribution of data. The Mann-Whitney U test or Student's t-test was used to compare continuous variables between the groups. Chi-squared test or Fisher's exact test was used for categorical variables. Intra and interobserver variability for ECG parameters was analyzed in 30 randomly selected patients using the Bland-Altman method. Data are expressed as percentages for categorical variables and as mean ± SD for parametric variables. Statistical significance was considered when a p-value <0.05.

RESULTS

The ST-segment depression in lead aVR on the admission ECG was used to divide patients into two groups. The mean age of all patients was 62,1±12,4 and 77 (26%) patients were female. STD in lead aVR was detected in 51 patients. Patients with STD in lead aVR (group 1) were more likely to be male, and thirty-eight patients (74%) showed significant STD in lead aVR. One hundred and sixty-four (57%) patients had hypertension, 88 (30%) had diabetes mellitus, 187 (65%) were smokers, 8 (2%) had a family history, and 48 (16%) had hyperlipidemia. The most common coronary artery disease risk factor was smoking (65%), followed by hypertension (57%), diabetes mellitus (30%), hyperlipidemia (16%), and family history (2%).

Demographic and laboratory parameters of the patients are shown in Table 1. HT was higher in group 1. Group 1 had a lower and statistically significant left ventricular ejection fraction than the other group. Glucose, LDL, and mean platelet volume were higher in group 1. Peak cTNI was higher in group 1 compared to the other group ($p<0.001$).

The comparison of angiographic and electrographic parameters between groups is shown in Table 2. The IRA was RCA in 260 patients. In group 1, RCA was the majority of IRAs (44/86%) and CX was the IRA for seven patients. The three vessel disease rate was higher in group 1. Group 1 had lower MBG and TIMI flow than the other group. Group 1 had a larger RCA diameter and a longer lesion length than the other group.

STR was not observed in 45% of patients in group 1, and it was observed at a higher rate than in the other group. Furthermore, 76% (n:39) of patients in group 1 had STD in V1-2 leads. The rate of using tirofiban was higher in group 1. The transient complete atrioventricular block was observed in 11.6% of group 1 patients and was statistically significant.

The Student's t-test was used to compare continuous variables between the groups. Categorical variables were compared using a chi-squared test.

The Student's t-test was used to compare continuous variables between the groups. Categorical variables were compared using a chi-squared test.

DISCUSSION

To our knowledge, this is the first angiographic study to evaluate the relationship between STD in lead aVR and microvascular function in patients with inferior MI who underwent successful primary

Table 1. Demographic features and laboratory parameters of the patients.

	aVR lead depression (+) n:51	aVR lead depression (-) n:236	p
Age	63.1±12.5	62±12.4	0.56
Sex (F/M)	13/38	64/172	0.86
DM (n,%)	24 (47)	64 (27)	0.07
HT (n,%)	40 (78)	124 (52)	0.001
Smoking (n,%)	35 (68)	152 (64)	0.62
Family history (n,%)	4 (7)	4(1)	0.03
Glucose, mg/dL	183±87	157±83	0.04
LDL, mg/dL	120±33	113±21	0.04
White blood count, 10'9 /L	13.5±11.3	11.3±3.4	<0.001
Hematocrit, g/dL	44.6±7.1	42.5±5	0.006
Platelet count, 10'9 /L	255±62	256±61	0.91
Mean platelet volume, fL	10.9±1.2	9.7±1.1	<0.001
Peak Tn- I (ng/ml)	23.4±4.5	14.1±7.8	<0.001
Creatinine (mg/dL)	1.1±0.2	0.9 ±0.4	0.51
GFR	75.7±16	79±14	0.07
EF(%)	46.6±5.1	53.4±5	<0.001

Abbreviations: Tn-I: troponin, F: female, M: male, LDL: Low-density lipoprotein, MI: myocardial infarction, HT: hypertension, DM: diabetes mellitus, EF: ejection fraction, GFR: glomerular filtration rate.

Table 2. The comparison of angiographic and electrographic parameters between groups.

	aVR lead depression (+) n:51	aVR lead depression (-) n:236	p
Pain to balon time, hours	3.67±0.91	2.61±1.4	<0.001
Multivessel disease, n (%)			
1 vessel	12 (23.5)	88 (37.2)	0.12
2 vessels	26 (50.9)	108 (45.7)	0.11
3 vessels	13 (25.4)	40 (16.9)	0.04
Infarct-related artery, n (%)			
RCA	44 (86.2)	216 (91.5)	<0.001
CX	7 (13.7)	20 (8.5)	
Glycoprotein IIb/IIIa antagonist, n (%)	43 (84.3)	56 (23.7)	<0.001
ST-segment depression ≥ 0.1mv in V1-V2	39 (76.4)	108 (45.7)	<0.001
Complete heart block	6 (11.7)	-	<0.001
STR < 70%	28 (54.9)	-	<0.001
STR > 70%	23 (45)	236	
TIMI Flow	2.58±0.6	2.98±0.1	<0.001
Myocardial blush grade	2.03±0.5	2.91±1.3	<0.001
Stent diameter (mm)	3.6±3	3.02±0.4	0.008
Stent length (mm)	28.6±13.5	23.9±9.8	0.006

Abbreviations: Tn-I: troponin, F: female, M: male, LDL: Low-density lipoprotein, MI: myocardial infarction, HT: hypertension, DM: diabetes mellitus, EF: ejection fraction, GFR: glomerular filtration rate.

PCI. In the present study, MBG and TIMI flow, which are indicators of microvascular function, were lower in patients with STD in lead aVR.

Acute MI is the leading cause of heart failure, arrhythmia, and mortality in patients with cardiovascular disease. Impaired microvascular perfusion is a significant prognostic factor in patients treated with primary PCI following an acute MI. Inflammation and mediators secreted by platelets and leukocytes cause vascular damage and lead to impairment in microvascular function

[14,15]. In our study, mean platelet volume and white blood count were higher in patients with STD in lead aVR compared to other group.

In patients with acute MI, Karahan et al. discovered that a longer QRS duration after primary PCI tended to reflect the presence of decreased microvascular perfusion [16]. A previous study demonstrated that good myocardial perfusion after primary PCI was associated with increased survival [17,18]. Our study demonstrated that STD in lead aVR was related to poor myocardial perfusion in patients with inferior MI. In other words, compared with patients without STD in lead aVR, patients with STD in lead aVR showed less ST-segment resolution and worse myocardial blush. Although Nair et al. found that STD in lead aVR was more frequent in CX-related inferior MI than RCA-related inferior MI [4]. In our study, STD in lead aVR was higher in RCA-related inferior MI patients. Menown et al. reported that STD in lead aVR may be a reciprocal alteration due to ST-segment elevation in the apical and inferolateral walls. The blood flow to these myocardial segments is provided by the large posterolateral branch of LCX or the AV branch of RCA [3]. Yumiko et al. also observed that STD in lead aVR predicted Cx or RCA infarction with a large posterolateral branch [19]. In our study, the higher frequency of STD in lead aVR in RCA-related inferior MI could be explained by the greater diameter and length.

Iwakura et al. showed that large myocardial ischemia and infarction before re-canalization were both associated with the occurrence of the no-reflow phenomenon [20]. Manohara et al. suggest that STD in lead aVR may indicate a large IRA for acute MI [21]. In addition, Kosuge et al. reported that the presence of STD in lead aVR after primary PCI in the inferior MI was associated with impaired perfusion [22]. The large diameter of the vessel and the longer lesion associated with MI may increase the thrombus load, which may cause more impact on the myocardium and lead to STD in lead aVR. In our study, we found higher troponin values and lower ejection fractions in patients with STD in lead aVR compared to those without. In addition, in patients with STD in lead aVR, RCA diameter was larger and lesion length was longer than in the other group. These findings may demonstrate that patients with this ECG change have larger infarct sizes and are prone to more myocardial damage.

This study had a few limitations. The sample size was relatively small, and the follow-up time for cardiac complications was short. MBG assessment is a visual assessment method that is not quantitative. In addition, there was no evaluation of right-sided precordial leads (V4R), which help identify the culprit artery in the inferior MI. We did not use a thrombus aspiration device.

CONCLUSION

In our study, STD in lead aVR was associated with impaired myocardial perfusion in patients with inferior MI. Patients with inferior MI and STD in lead aVR are prone to worse angiographic outcomes and more myocardial damage. Therefore, effective treatment and follow-up should be provided to these patients.

Author contribution

Study conception and design: BA and MZK; data collection: BA; analysis and interpretation of results: MZK; draft manuscript preparation: BA. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the ethics committee of our hospital (Protocol no: 684 Date: 12/02/2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

The Factors Related to the 6 Minute Walk Test: The Experience of a Referral Lung Transplantation Center

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ABSTRACT

Objectives: The 6-minute-walk test is a practical and widely used test, which indicates the exercise capacity in patients with a severe pulmonary disease. The study aims to investigate the related factors with the 6-minute-walk test in lung transplantation candidates.

Materials and Method: The data were collected retrospectively from 349 patients, between January 2012 and September 2020. The patients were grouped according to their underlying lung disease as obstructive lung diseases, interstitial lung diseases, and infective lung diseases. The data collected included patient demographics, gender, body mass index, artery blood gas, the results of the respiratory function test, six-minute walk test, long-term oxygen therapy, and the need for non-invasive mechanical ventilation [such as group 1 (6MWD<200) and group 2(6MWD≥200)]. All of the collected data were analyzed and compared between the groups.

Results: Overall, 349 patients were included in the study, and there were 123 females and 226 males (35.2% and 64.8% respectively) with a mean age of 46.92 ± 14.1 years. Their mean body mass index was 23.58 ± 12.52 kg/m2, the median FEV1(%) was 35.3 (33.4-37.2), the median six-minute walk distance was 222 m (125-335 m), and the mean PaO2/FiO2 (P/F) was 250.32% ± 74.81, the mean PCO2 was 45.71 mmHg ± 11.97. Furthermore, the patients using long-term oxygen therapy were (n=274, 78.5%) and non-invasive mechanical ventilation were (n=125, 35.8%). The mortality status, P/F, long-term oxygen therapy usage, and non-invasive mechanical ventilation usage were different between Group 1 and Group 2 (p=0.001, p=0.001, p<0.001, and p<0.001, respectively). There was no difference between the groups in patients with and without IPF between underlying diseases. The 6-minute walk test was found to have moderate correlation with FEV1 and P/F; and a negative correlation with age and PCO2 (p<0.01, r=0.33.8, p<0.001, r= 38.1 and p=0.17, r=12,7, p<0.001, r=-0.30.6, respectively). There was no correlation between P/F, FEV1, and body mass index; and also, between PCO2, age, and body mass index. Age had a weak correlation with FEV1(p<0.001, r=19.3). There was no correlation between the age and 6MWD, as well as P/F, PCO2, and the body mass index. The factors affecting survival in multivariate analysis were investigated by using the Cox regression model. It was observed that gender (OR, 0.001; 95% CI, 0.246-0.716; p=0.42), FEV1(OR, 1.02; 95% CI, 1.00-1.04; p<0.001), P/F (OR, 1.00; 95% CI, 1.00-1.01; p<0.001), and LTOT (OR, 9.83; 95% CI, 3.70-26.14; p<0.001) were independent factors associated with 6MWD<200 m.

Conclusion: The 6-minute walk test is associated with mortality, gender, poor oxygenation, and with the utilization of domiciliary non-invasive mechanical ventilation or long-term oxygen therapy. Furthermore, it is an independent risk factor for mortality in lung transplant candidates and in providing a valuable method for the management of patients.

Keywords: Lung transplant candidates, six minute walk test, end stage lung diseases

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INTRODUCTION

The 6-minute walk test (6MWT) is a useful measure of functional capacity. Furthermore, for patients with pulmonary and cardiac disease, it can be widely used for measuring the outcomes before and after treatment, their functional status evaluation, as well as for predicting mortality and hospitalization [1]. This test is performed by monitoring the patient's heart rate and oxygen saturation with a pulse oximeter device, accompanied by a trained respiratory physiotherapist. The test is based on the principle of walking a 50-meter corridor for a duration of six minutes. It is also a practical test that the patient can easily apply. In order to standardize the performance of the patient, encouraging expressions should be used in one minute intervals, as per the recommendations in the ATS Guidelines, since the patient's performance is affected by these encouraging phrases [1].

The previous studies have shown that 6MWT results predict complications, prolonged hospital stays, and mortality after cardiothoracic surgery [2-4]. In the study by Chaikriangkrai et al. conducted with lung transplant patients, they showed that the 6-minute walking distance (6MWD) is an independent predictor for postoperative mortality [5]. However, it has been observed that the data from the studies, which have been conducted to identify optimal thresholds for recognizing and distinguishing high-risk lung transplant candidates based on exercise tolerance, have been found to be insufficient [6]. Accordingly, the objectives of this study were to investigate the association of 6MWD with clinical characteristics and mortality, thus investigating its role in identifying the lung transplant candidates under risk.

MATERIALS AND METHODS

Overall, 349 patients with end-stage lung disease were selected for this single-center retrospective cohort study, which was conducted in a tertiary hospital that is also the main referral lung transplantation center in the country, from January 2012 to October 2020.

This study was approved by the local ethics committee of the Kosuyolu Specialty Training and Research Hospital Istanbul- 24.08.2021 No: 2021/10/521). The ethical approval was given in accordance with the Declaration of Helsinki.

Patients

The patient group included in the study were those who were older than 18 years of age, had end-stage lung disease, and were referred to a lung transplant center for treatment. Patients with a physical or orthopedic disability that would prevent walking, recent MI or acute coronary disease, systolic heart pressure above 180 mmHg, insufficient data, and seventy-four transplants were excluded from the study.

Data collection

The data were collected from the files of the patients and the operating systems of the hospital and subsequently analyzed using IBM SPSS Statistics for Windows v.23.0 IBM Corp. Released 2015. The patient demographics, gender, body mass index (BMI), artery blood gas, respiratory function test, 6MWD (Group 1≥200 m, Group 2<200 m), long term oxygen therapy (LTOT), NIMV (non-invasive mechanical ventilation) need, as well as the time since diagnosis results were recorded. The values were calculated by using mean and standard deviation values, and the median and inter-quartile ratios according to their distribution. Accordingly, while the median and inter-quartile ratios were used for non-parametric variables; mean and standard deviation were used for the parametric variables. The categorical variables were compared with the Chi-Square test. The correlations of the collected parameters were determined using Pearson's correlation coefficient(r). Receiver operating characteristics (ROC) analysis was performed to calculate the cut-off value of the six-minute walking test distance. The candidate variables were chosen based on a p-value<0.25 from the univariate logistic regression. Backward elimination was performed with those variables. The results of the final logistic regression models were represented with the odds ratio (OR), a confidence interval of 95%, and the p-value. The level of statistical significance was set at a p-value < 0.05. All reported p-values were 2-sided.

These patients were categorized as per their disease type into OLD (obstructive lung diseases), ILD

(interstitial lung diseases), and SLD (Suppurative Lung diseases). Their demographic profile was noted; 6MWT and spirometry were conducted as per the ATS guidelines at the initiation of the study [1].

Six-minute walk and pulmonary function tests

The 6MWT was performed indoors, along a flat and straight 50 m corridor supervised by a trained researcher, according to the ATS guidelines. A prior practice 6MWT was not performed. All 6MWT were performed with pulse oximetry for continuous recording of the oxygen saturation (SpO2) level. Dyspnea was assessed using the Borg scale for each minute during the 6MWT and the maximum dyspnea level was recorded. Patients were encouraged every minute using the standardized recommended sentences: "you are doing well" or "keep up the good work". The patients were allowed to stop during the test; but were instructed to resume the walking as soon as they felt able to do so. All the pulmonary function data were obtained as absolute values and expressed as % predicted of reference values according to the ATS/ERS consensus guidelines [1,7].

RESULTS

Overall, 349 patients were included in the study, there were 123 females and 226 males (35.2% and 64.8%; respectively) with a mean age of 46.92 ± 14.15 years. Their mean BMI was 23.58 ± 12.52 kg/m2, median FEV1 was 35.3 (IQR, 33.4-37.2%), median 6MWD was 222 m (IQR, 125-335 m). The (arterial blood oxygen tension (PaO2)/fraction of inspired oxygen (Fio2) ratio) P/F was 250.32% ± 74.81, mean PCO2 was 45.71 mmHg ± 11.97, patient using LTOT (n=274, 78.5%) and NIMV (n=125, 35.8%). The median time since diagnosis was 6 years (IQR, 3-10 years). The demographic characteristics and clinical parameters of the patients have been summarized in Table 1. The underlying diseases of the patients, who applied as lung transplant candidates, were idiopathic pulmonary fibrosis (n = 123, 35.2%), chronic obstructive pulmonary disease (n = 71, 20.3%), bronchiectasis (n = 64, 18.3%), cystic fibrosis (n = 27, 7.7%), interstitial lung disease (n = 16, 4.6%), sarcoidosis (n=10, 2.9%), silicosis (n = 18, 5.2%), retransplant (n=2, 0.6%), bronchiolitis obliterans (n = 4, 1.1%), histiocytosis X (n = 3, 0.9%), idiopathic pleuroparenchymal fibroelastosis (n = 3, 0.9%), interstitial pneumonia due to bleomycin (n = 2, 0.6%), lymphangioleiomyomatosis (n = 2, 0.6%), hypersensitivity pneumonia (n = 2, 0.6%), and alveolar microelastosis (n = 2, 0.6%). The patients' demographics such as gender were found to have a significant difference between the groups (p=0.04). Other demographics were similar in both groups.

The mortality status, P/F, LTOT usage, and NIMV usage were different between Group 1 and Group 2 (p=0.001, p=0.001, p<0.001, and p<0.001, respectively) There was a difference between the groups in patients with and without OLD (Table 2).

Cut-off Value of Six-minute Walk Distance

In the evaluation of lung transplantation candidates in end-stage lung patients, the appropriate cut-off

Table 1. Demographic and Clinical Parameters of the Study Groups.

	All patients (n=349)	OLD (n:74)	ILD (n:185)	SLD (n:90)	P value
Age, mean ± SD	46.92±14.15	55.15 ± 10.6	48.5 ± 13.4	36.9 ± 12.3	<0.001
Sex, female, n (%)	123(35.2%)	15(20.2%)	66(35.6%)	42(39.5%)	0.002
Time since disease, median (IQR, 25-75%)	8.10(7.36-8.84)	7.66(6.35-8.96)	5.19 (4.55-5.84)	14.43(12.68-16.18)	<0.001
BMI, mean ± SD	23.58± 12.52	23.6 ± 4.1	23.5± 4.6	23.6 ± 4.8	0.997
$PaCO_2$, mean ± SD	45.71 ± 11.97	50.4±11.7	45.8±13.1	42.5±10.4	<0.001
PaO_2 /FiO_2, mean± SD	250.32 ± 74.81	250.4±74.3	253.2±76.6	244.2±71.7	0.648
6MWD, meters, median (IQR, 25-75%)	222 (125-335	226(196-255)	219(197-241)	211(176-247)	0.230
FEV_1, %, median (IQR, 25-75%)	35.3 (33.4-37.2)	27.2(24.1-30.2)	42.2(39.9-45.0)	27.7(25.3-30.1)	<0.001
NIMV,n,%	125(79.1%)	46(62.1%)	21(54.5%)	58(64.4%)	<0.001
LTOT, n, %(>12 hours)	274(78.5%)	63(85.1%)	141(76.2%)	70(77.7%)	0.284

Abbreviations: OLD: Obstructive lung disease; ILD: Interstitial lung disease; SLD: Suppurative Lung diseases; PaCO2:partial arterial pressure of carbon dioxide; PaO2:partial arterial pressure of oxygen; FiO2: fraction of inspired oxygen; BMI: body mass index; 6MWD:6-minute walking distance; FEV1: Forced Expiratory Volume in the First Second, LTOT: Long term oxygen treatment; NIMV: Non-invasive mechanical ventilation; SD: standard deviation, IQR:Interquartile ratio

Table 2. Comparison of Age, Gender, BMI, Underlying diseases, Mortality, P/F, LTOT, NIMV in patients with Group 1 and Group 2.

	≥200 m (Group1)	<200 m (Group 2)	P value
Age, IQR (25-75%)	52 (37-61)	48 (34-58)	0.114
Gender, (n, %)			
Female	59 (29.1)	64 (43.8)	**0.004**
BMI, (n, %)			
≤18	29 (45.3)	35 (54.7)	0.592
>18	117 (41.1)	168 (58.9)	
ILD (n,%)	70 (37.8)	115(62.2)	0.108
SLD (n,%)	53 (58.9)	37 (41.1)	0.902
OLD (n,%)	35 (47.3)	39 (47.3)	**0.035**
Mortality, (n, %)	83 (40.9)	85 (58.2)	**0.001**
P/F, (n, %)			
≥237	86 (42.4)	88 (60.3)	**0.001**
>237	117 (57.6)	58 (39.7)	
LTOT, (n, %)	133 (65.5)	141 (96.6)	**<0.001**
NIMV, (n, %)	56 (27.6)	69 (47.3)	**<0.001**

Abbreviations: OLD: Obstructive lung disease; ILD: Interstitial lung disease; SLD: Suppurative lung diseases; (P/F) PaO_2:partial arterial pressure of oxygen; FiO_2: fraction of inspired oxygen; BMI: body mass index; 6MWD: 6-minute walking distance; LTOT: Long term oxygen treatment; NIMV: Non-invasive mechanical ventilation; LTOT: Long term oxygen treatment; NIMV: Non-invasive mechanical ventilation, IQR:Interquartile ratio; m: meter

Table 3. Logistic regression analysis for Mortality.

	Univariate logistic regression			Multivariate logistic regression		
	Odds Ratio	Confidence Interval (95%)	P value	Odds Ratio	Confidence Interval (95%)	P value
Age	1.01	0.99-1.02	0.087	1.02	1.00-1.03	0.024
Gender	1.33	0.86-2.07	0.195	1.41	0.88-2.26	0.145
BMI	0.97	0.93-1.01	0.224	0.95	0.91-1.00	0.099
FEV_1, %	0.99	0.98-1.01	0.906	1.01	0.99-1.02	0.063
6MWD(<200m)	2.01	1.30-3.10	0.001	1.64	1.01-2.64	0.042
LTOT	0.53	0.31-0.89	0.019	0.86	4.89-35.09	0.649
NIMV	0.61	0.39-0.95	0.029	0.70	0.42-1.14	0.155

Abbreviations: BMI: body mass index; 6MWD: 6-minute walking distance; FEV_1: Forced Expiratory Volume in the First Second, LTOT: Long term oxygen treatment; NIMV: Non-invasive mechanical ventilation.

value for the 6MWD was 200 m in the prognosis. The sensitivity of this cut-off value was 53.6% and specificity was 64.9%, and the area under the curve was (AUC) 0.610 (95% CI, 0.551-0.669, p<0.001) (Figure 1).

The distribution of 6MWD values according to the 200-threshold value in the underlying disease groups has been shown in Figure 2.

6MWD value was found to have a moderate correlation with FEV1 and P/F; and a negative correlation with age and PCO2 (p<0.01, r=0.33.8, p<0.001, r= 38.1 and p=0.17, r=12,7, p<0.001, r=-0.30.6, respectively). There was no correlation between P/F and FEV1, BMI; as well as between PCO2 and age, BMI. The relationship between FEV1 and baseline values of 6MWD has been shown in Figure 3.

In the logistic regression analysis, the univariate predictors were age, gender, BMI, 6MWD (<200m), patients receiving long-term oxygen therapy, and patients using NIMV. The multivariate analysis identified age (OR, 1.02; 95% CI, 1.00-1.03; p=0.024), and 6MWD(<200m) (OR, 1.64; 95% CI, 1.01-2.64; p=0.042) as the independent predictors of mortality (Table 3).

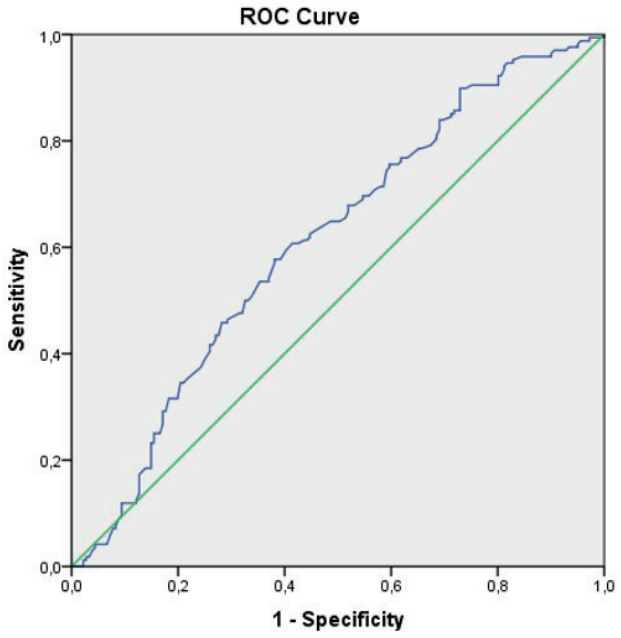

Figure 1. ROC curve analysis of six minute walk distance for predicting of prognosis in lung transplant candidates.

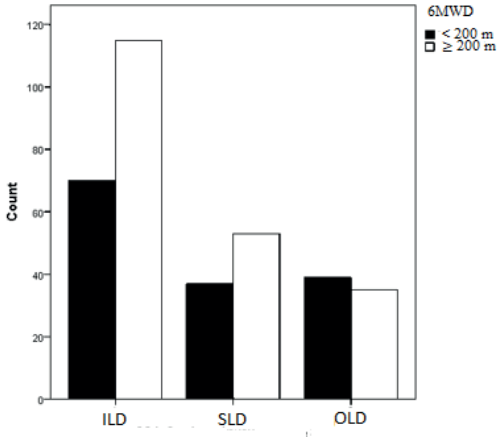

Figure 2. The proportion of underlying disease groups for six minute walk distance by 200 m threshold.

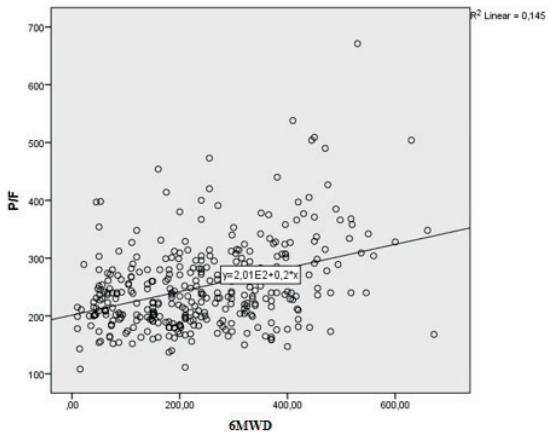

Figure 3. Correlation between baseline values of six minute walk distance and Pa02/FiO2 ratios'.

DISCUSSION

The study found that a low 6MWD value in lung transplant patients was associated with mortality, female gender, OLD as the underlying disease, poor oxygenation, and the use of respiratory failure devices (NIMV and LTOT). The results also demonstrated that age and low 6MWD are independent predictors of mortality. Therefore, our primary results reveal that the 6MWT is a practical and beneficial method that can guide the clinician in distinguishing critical lung transplant candidates and in assisting patient management. The previous studies have shown that 6MWT is a good indicator of exercise capacity, which can predict postoperative survival in lung transplant patients. Thus, this test has been included in pretransplant scoring systems and in the pretransplant evaluation process [8].

With this measurement, the survival prediction has been shown in chronic lung diseases such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and pulmonary hypertension (PHT) [9,10]. In addition, 6MWD was found to be associated with waiting-list mortality in lung transplant candidates with end-stage lung disease [11].

Lederer et al. found that a 6MWD of less than 350 meters in patients with IPF, who were evaluated for lung transplantation, was associated with a shorter survival time. On the other hand, in a cohort study conducted in patients with newly diagnosed IPF, no relationship was found between the 6MWD and survival [12-14]. In addition, another study with 9525 lung transplant candidates reported that 6MWD was strongly associated with postoperative survival [6]. In the study conducted by Chaikriangkrai et al. with 324 lung transplant patients, it was shown that the preoperative 6MWD is an independent predictor for postoperative mortality, similar to the results of our study [5]. In the same study, it was shown that a low 6MWD (<237 feet) was associated with underlying OLD disease, the need for preoperative oxygen or ECMO, double lung transplantation, high LAS score, postoperative vasopressor requirement, and mortality [5].

Similarly, in our study, we found that transplant candidates with underlying OLD disease and patients with poor oxygenation (P/F ratio below 237) had a lower 6MWD. OLD patients in our study were observed to be the group of patients

who were treated more frequently with LTOT and had the highest mean PCO2 value in blood gas disorders. Therefore, a significant portion of the use of NIMV consisted of OLD patients. The low 6MWD associated with NIMV, LTOT, and low oxygenation can be explained by this situation.

We found 6MWD(<200m) and age to be the predictors of mortality. In our study, in the multivariate analysis; while the 6MWD was found to be a predictor of mortality, FEV1 was found to have no effect. The 6MWT result aids in planning such as estimating mortality, management of end-stage lung disease, and deciding referral for lung transplantation. Similarly, in the study by Karanth et al. on 139 patients with chronic lung diseases, 6MWD was found to be a better predictor of mortality than FEV1 [15]. Pinto-Plata VM concluded in his study that 6MWD is an independent predictor for mortality in COPD [16].

Dajczman E et al. found that the survival rate in COPD patients with a 6MWD value less than 150 meters at baseline was 58% [17]. Du Bois RM et al. reported that a 50 m per year decrease in 6MWD was associated with a four-fold increase in one-year mortality in IPF [18]. As a mortality predictor, Karanth et al. determined a cut-off value of 240 m in 6MWD, while we found a cut-off value of 200 m in our study [15]. Tuppin et al. showed that patients with a six-minute walking distance below 315 meters had a higher risk of mortality [19].

Pulmonary function test may be a relatively inadequate tool to measure the functional status in patients, especially since our patient population, which have been accepted for evaluation for lung transplantation in our clinic, consists of patients with end-stage lung disease accompanied by severe respiratory failure and difficulties in performing spirometry. In addition, 6MWT can be easily applied even in patients with advanced respiratory failure.

Our study shows that 6MWT is an important tool in addition to spirometry in evaluating end-stage lung patients as lung transplant candidates. This study supports the use of the 6MWT as a guide to assess the mortality in patients with end-stage lung disease.

In conclusion, this study found that the 6MWD was associated with mortality, gender, underlying lung disease, oxygenation status, and the use of home-type chronic respiratory failure devices (NIMV and LTOT) in lung transplant candidates. Furthermore, the study has demonstrated that age and a short 6MWD are independent risk factors for mortality. In lung transplant candidates, the 6MWT is a practical and useful test that can guide the clinician in distinguishing critically ill patients; therefore, it constitutes an important component of patient management.

Author contribution

Study conception and design: PAG; data collection: PAG; analysis and interpretation of results: PAG; draft manuscript preparation: PAG. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Koşuyolu Higher Specialized Educational and Research Hospital Clinical Research Ethics Committee (Protocol no. 2021/10/521/24.08.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Distribution of Intestinal Parasites Detected in Ankara Training and Research Hospital between 2017 and 2020

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ABSTRACT

Objective: The aim of this study was to evaluate the current prevalence of intestinal parasites in patients admitted to Ankara Training and Research Hospital during the period of 2017 to 2020.

Materials and Methods: Intestinal parasitic examination results of patients between 2017 and 2020 were evaluated retrospectively. Data on demographic and clinical parameters were obtained from the laboratory information management system.

Results: E. vermicularis eggs were found in 7.2% of 2348 samples examined by cellophane tape method. One or more intestinal parasites were detected in 18.2% of 4211 samples examined stool concentration method. Intestinal parasite positivity was the highest in children aged 6-18 years among age groups. The most frequently detected intestinal parasites were Blastocystis sp., E. vermicularis, Dientamoeba fragilis and Giardia intestinalis.

Conclusion: Although our hospital is in the center of Ankara Training and Research Hospital, it mostly serves patients with low socioeconomic status and immigrant individuals; therefore total intestinal parasite detection rate was found relatively high. Intestinal parasitic infections are still an important public health issue in our country. It is important to determine the prevalence of parasitic infections to develop optimal prevention and treatment strategies.

Keywords: Parasites, *blastocystis*, giardia, *dientamoeba*

INTRODUCTION

Intestinal parasitic infections continue to maintain their importance as a common public health problem, especially in countries with low socioeconomic status. Low level of education, inadequate sanitation of drinking water and food, and lack of attention to personal hygiene are the main factors that increase the prevalence of intestinal parasitic infections [1].

Although intestinal parasites are common, the often non-acute course of the parasitic infections may cause clinicians to skip the diagnosis [2]. However, intestinal parasitic infections are of great importance, especially in children, as they can cause malnutrition, anemia, growth and developmental delay, deterioration in cognitive skills and decrease in school success [3,4]. At the same time, intestinal parasites can cause serious and widespread infections that can be life-threatening in immunocompromised patients [5].

The most common method for the diagnosis of gastrointestinal parasites is direct microscopic examination (native-lugol). The application of concentration methods in the examination of intestinal parasites increases the chance of diagnosis, especially in stool samples with low parasite loads [7].

In this study, we aimed to determine the prevalence of protozoa and helminths in the samples sent to Ankara Training and Research Hospital Medical Microbiology Laboratory for the purpose of examination of intestinal parasites between 2017-2020 and to evaluate the distribution of these parasites according to symptoms, age, clinics and nationality.

MATERIALS AND METHODS

Ethics Committee Approval

Ethics committee approval dated 06.07.2021 and numbered E-21-644 was obtained from Ankara Training and Research Hospital Clinical Research Ethics Committee.

Selection of Patient Groups

In the study, patients who applied to our hospital with gastrointestinal complaints and asked for intestinal parasitic examination via cellophane tape method and stool concentration method by the clinicians during the three-year period between 2017 and 2020 were evaluated retrospectively. Data on demographic and clinical parameters of patients were obtained from the laboratory information management system.

Parasitological Examinations

Fresh stool samples taken into a commercial fecal concentration tube with fixative solution (Parasep® Fecal Parasite Concentrators, Apacor, USA) were delivered to the laboratory and the sediment obtained after centrifugation was examined by native-lugol microscopy to detect intestinal parasites in stool samples [8,9]. Entamoeba spp., Dientamoeba fragilis etc. suspected specimens were stained with the Wheatley trichrome staining method [10].Cryptosporidium spp. suspicious samples were stained with the Modified Kinyoun acid-fast staining method [11]. Cellophane tape method was used to detect Enterobius vermicularis eggs [12].

Statistical Analysis

Statistical analysis was performed using SPSS 23 (IBM Inc, New York, USA). Chi-square test was used to compare the gender, age group distributions and Turkish citizenship status between positive and screened cases, and $p<0.05$ was considered statistically significant. Descriptive statistics were given as percentage and frequency.

RESULTS

Results of Cellophane Tape Method

A total of 2348 patients were evaluated for E. vermicularis eggs by the cellophane tape method. Of these patients, 1169 (49.8%) were males and 1179 (50.2%) were females, with a mean age of 11.99 (0-81) and 13.05 (1-79), respectively.

E. vermicularis eggs were found in 169 (7.2%) of 2348 samples and Taenia spp. eggs were found in four samples (0.2%). Among patients with E. vermicularis, 88 (52.1%) were males, with a mean age of 9.53 (1-51) and 81 (47.9%) were females, with a mean age of 11.91 (3-73). The distribution of these patients by age, gender and nationality is given in Table 1. When the distribution of patients with E. vermicularis was examined in terms of symptoms/clinical diagnosis, abdominal pain was observed in 44 (26%), parasitic infection was suspected in 35 (20%), and gastroenteritis was observed in 16 (9%) patients.

Results of Stool Examination

Stool samples of 4211 patients were evaluated with native-lugol microscopic examination after concentration method. Of these patients, 2127 (50.5%) were males, with a mean age of 11.56 (0-90), 2084 (49.5%) were females, with a mean age of 12.61 (0-85). One or more intestinal parasites were detected in 765 (18.2%) of 4211 samples.

Among the patients in whom intestinal parasites were detected, 394 (51.5%) were males, with an average age of 13.1 (0-76), and 371 (48.5%) were females, with an average age of 13.8 (1-78). The distribution of these patients by age, gender and nationality is given in Table 2, and their distribution in terms of symptoms/clinical diagnosis is given in Figure 1.

Blastocystis sp. was the most common intestinal parasite and detected in 611 (14.5%) of the stool samples. Wheatley trichrome stain was applied to the suspicious samples that were examined by the concentration method and D. fragilis was detected in 119 (2.8%), Entamoeba histolytica/dispar in

Table 1. Distribution of samples examined by cellophane tape method according to age, gender and nationality.

	E. vermicularis positivity (n/%)	Total number of samples examined (n/%)	p value
Gender:			
Female	81 (48%)	1179 (50.2%)	0.538
Male	88 (52%)	1169 (49.8%)	
Age:			
<6	32 (18.9%)	636 (27.1%)	
6-18	**124 (73.4%)**	1360 (57.9%)	**0.000***
19-39	9 (5.3%)	195 (8.3%)	
≥40	4 (2.4%)	157 (6.7%)	
Nationality:			
Turkish citizen	160 (94.7%)	2223 (94.7%)	0.679
Other	9 (5.3%)	125 (5.3%)	
TOTAL	**169 (%7.2)**	**2348**	

*p<0.05

Table 2. Distribution of stool samples examined for intestinal parasites by age, gender and nationality.

	Number of samples with intestinal parasites (n/%)	Total number of samples examined (n/%)	p value
Gender:			
Female	371 (48.5%)	2084 (49.5%)	0,244
Male	394 (51.5%)	2127 (50.5%)	
Age:			
<6	140 (18.3%)	1337 (31.7%)	
6-18	**534 (69.8%)**	2396 (56.8%)	**0,012***
19-39	48 (6.3%)	235 (5.8%)	
≥40	43 (5.6%)	243 (5.7%)	
Nationality:			
Turkish citizen	626 (16.3%)	3831 (91%)	0,240
Other	139 (36.6%)	380 (9%)	
TOTAL	**765 (%18.2)**	**4211**	

*p<0.05

15 (0.3%) of the samples. With Modified Kinyoun acid-fast staining method, Cryptosporidium spp. was detected in one patient. The distribution of intestinal parasites detected by the concentration method is given in Figure 2.

DISCUSSION

Intestinal parasitic infections are important public health problem, especially in underdeveloped and developing countries with low socioeconomic status. Although many preventive strategies have been implemented to control these infections, the methods used to determine the prevalence of intestinal parasites are insufficient. It is important to use advanced techniques in order to accurately determine the prevalence of intestinal parasites and to develop effective control strategies [13].

In our study one or more intestinal parasites were found in 18.2% of the stool samples examined by the concentration method. Native-lugol direct microscopic examination is the most commonly used method for the detection of intestinal parasites, as it is a fast and easily applicable method. However, the sensitivity of this method is low, and the chance of diagnosis decreases, especially in samples that are not delivered to the laboratory immediately [14]. Taking the stool sample into the fixative and delivering it to the laboratory and applying the concentration method to the stool increase the chance of diagnosis of intestinal parasites [15].

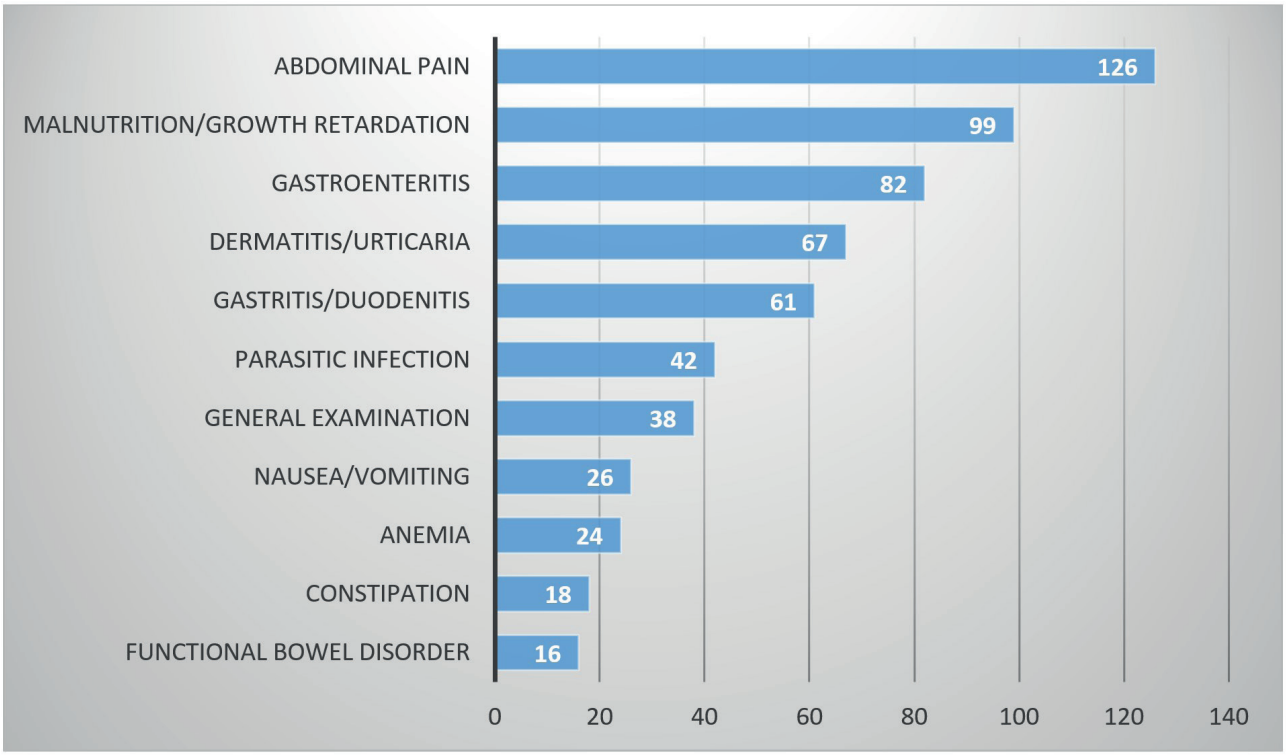

Figure 1. Distribution of patients with intestinal parasites detected in stool samples in terms of symptom/clinical diagnosis.

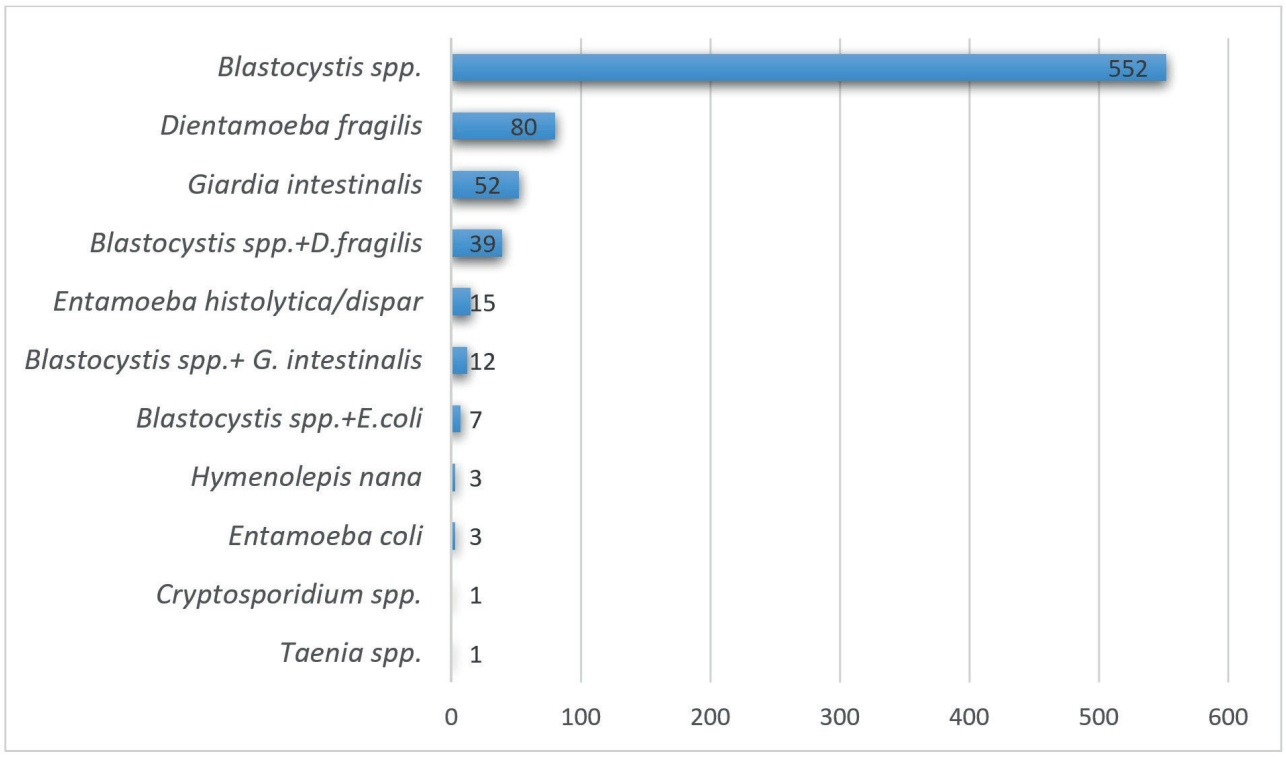

Figure 2. Distribution of intestinal parasites detected in stool samples.

The prevalence of intestinal parasites in our country varies according to the methods used in the studies and the region where the study was conducted. In the study conducted by Cengiz et al. in Van, in which 11-year retrospective data were analyzed, one or more intestinal parasites were detected in 34.1% of 69633 patients. The most common parasites were Blastocystis sp., G. intestinalis, Entamoeba coli and Ascaris lumbricoides [16]. In the study conducted by Öncel, intestinal parasites were observed in

31.6% of 7353 stool samples examined in Şanlıurfa. The most frequently detected parasites were Blastocystis sp., E. coli and G. intestinalis [17]. In the study of Yula et al. in Mardin, intestinal parasites were observed in 27.6% of 1620 stool samples examined and G.intestinalis and Taenia spp. were most commonly detected parasites. This finding was attributed to the high consumption of raw meat in the region [18]. In the study conducted by Kırkkoyun Uysal et al. in Istanbul and examining 25-year data, intestinal parasites were found in 5% of the stool samples of 111889 cases. The most common parasites were G. intestinalis and E. vermicularis [19]. In the study conducted by Gülmez et al. in Ankara, 10-year data were analyzed and intestinal parasites were found in 4.2% of 85707 stool samples [20]. It is noteworthy that the prevalence of intestinal parasitic infections is quite high in regions with low socioeconomic status in our country. In our study, when cellophane tape and stool concentration methods were evaluated together, intestinal parasite positivity was found in 14.2% of 6559 patients examined over a three-year period. The higher intestinal parasite detection rates than in similar regions, is thought to be related to the low sociocultural level of patients.

In our study, the most frequently detected protozoa were Blastocystis sp. and D. fragilis, the most frequently detected helminths were E.vermicularis and Taenia spp. Blastocystis sp. and D. fragilis are intestinal protozoans whose pathogenicities are still controversial, despite their increasing incidences in recent years. Due to the difficulties in diagnosis by routine microscopic examinations, the true prevalence of D. fragilis is not well known. In our study, D. fragilis prevalence was found 2.8% by microscopic examination. In the recent studies conducted in Turkey, the prevalence of D. fragilis was found 11.9% and 12.04%, respectively, by molecular methods [21-23].

In our study, intestinal parasite positivity was two times higher among refugee/immigrant population than in Turkish citizens. Indeed, intestinal parasitic infections are reported more prevalent in refugees worldwide. In a study conducted in Denmark, the prevalence of G.intestinalis and Blastocystis sp. was found high in asymptomatic refugee population [24]. In another study in Thailand, pregnant women from the refugee camp were found two times more likely to be infected with soil-transmitted helminth infections [25]. In the current study soil-transmitted helminths (STH) were found in any of the patients, probably due to the non-endemic living areas for STH. In a study conducted in Canada, it is reported that refugees were at greater risk of parasitic infections with a high prevalence of intestinal parasites, like as our study indicate [26].

Although parasitic infections are mostly asymptomatic, it has been reported that these infections may trigger conditions such as diarrhea, malabsorbion, dyspepsia, irritable bowel syndrome or anemia [27]. In a study conducted in Turkey, it has been reported that Blastocystis sp. and D. fragilis might play a role in chronic urticaria and indicated that parasitic infections should not be neglected in patients with cutaneous manifestations [28]. But on the contrary, in another study in Iran, the prevalence of various parasites between case and control groups was not found significant [29]. In our study, it was observed that in addition to gastrointestinal complaints such as abdominal pain and gastroenteritis, non-gastrointestinal complaints such as malnutrition, growth retardation, dermatitis and urticaria were quite common in patients with intestinal parasites. It should be kept in mind that intestinal parasites may also be a factor in patients presenting with these complaints, as in asymptomatic patients.

Limitations of the study

Due to nature of the retrospective study, cellophane tape method and stool concentration methods could not be applied to all patients concurrently. Since the modified acid-fast staining method was not used in routine parasitological examinations, the prevalence of sporozoan parasites such as Cryptosporidium spp., Cyclospora cayetanensis and Cystoisospora belli could not be determined in our study. However, in order to determine the prevalence of these protozoa, it is important to apply the modified acid-fast method, especially in watery stool samples. Another limitation of the study was single-day examination of samples. For an ideal parasitological examination, at least three samples taken periodically should be examined.

CONCLUSION

In Turkey, intestinal parasitic infections are still an important public health issue. It has a great importance to determine the prevalence of parasitic infections to develop optimal prevention and treatment strategies. More studies with advanced diagnostic tests are needed to accurately determine the prevalence of intestinal parasites and to understand their pathogenic roles.

Author contribution

Study conception and design: FK and MK; data collection: MK; analysis and interpretation of results: FK and MK; draft manuscript preparation FK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Non Interventional Ethics Board/Committee (Decision number: E-21-644, 06/07/2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

Comparison of Favipiravir to Hydroxychloroquine Plus Azithromycin in the Treatment of Patients with Non-critical COVID-19: A Single-center, Retrospective, Propensity Score-matched Study

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ABSTRACT

Objectives: In this study, we compared the clinical outcomes and effects of the treatments on laboratory parameters between patients who were treated with favipiravir (FAV) or hydroxychloroquine plus azithromycin (HCQ/AZ) for COVID-19 pneumonia in non-Intensive Care Unit (non-ICU) patients.

Methods: We collected data of 260 moderate or severe COVID-19 patients hospitalized in COVID-19 wards between March 20, 2020, and September 30, 2020 retrospectively. We used propensity score matching to evaluate treatment effect on laboratory parameters of COVID-19 infection.

Results: We compared 42 patients using FAV and 42 HCQ/AZ after propensity score matching. While there were statistical differences between the therapy groups in terms of transfer to ICU and/or exitus before matching (p=0.031), this was not significant after propensity analysis (p=0.250). Patients treated with FAV stayed in the hospital nearly one more day than HCQ/AZ group but the difference was not statistically significant (9.02 days vs 8.14 days, p=0.903). The levels of AST,ALT, and LDH increased at discharge in both groups, especially in the FAV group.

Conclusions: FAV is not superior to HCQ/AZ in the treatment of COVID-19 infection in hospitalized patients with pneumonia.

Keywords: COVID-19, hydroxychloroquine, azithromycin, favipiravir, propensity-matched analysis

INTRODUCTION

After more than a year of the COVID-19 (Coronavirus Disease 2019) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with over 185,000,000 infected individuals and 4 million deaths worldwide [1], there is still insufficient evidence about the optimal treatment. Although vaccination programs have been launched in many countries, the rate of vaccination is far from taking the pandemic under control soon, and the number of cases is still increasing with a new challenge by variant strains [2]. Therefore, the necessity of defining an optimal treatment modality is essential than ever.

Since the beginning of the pandemic, several therapeutic agents have been administered in different countries. Despite in vitro effects of interferons, lopinavir/ritonavir, ribavirin, chloroquine (CQ), hydroxychloroquine (HCQ), remdesivir, favipiravir (FAV), and ivermectin, there is still no approved treatment with proven efficacy [3].

HCQ, alone or in combination with azithromycin (AZ), has being used for treatment of COVID-19 during the initial months of the pandemic worldwide when it was enlisted as an option for treatment due to its anti-inflammatory and antiviral effects [4-7]. After initial controversial reports on efficacy, the Solidarity Trial and Recovery Trial both revealed that HCQ did not reduce the mortality and duration of hospitalization of COVID-19 patients [8,9]. On the other hand, increased concerns for cardiovascular adverse events have precluded the widespread use of HCQ alone or combined with AZ [10].

FAV, an RNA-dependent RNA polymerase inhibitor, has been shown to inhibit SARS-CoV-2 infection in Vero E6 cells (EC50 value 61.88 μM) [11-13]. Although several observational studies have suggested that FAV is beneficial for improvement in thoracic computerized tomography (CT) and viral clearance, control inflammatory responses in patients undergoing mechanical ventilation, and shortening the length of stay in the intensive care unit (ICU) [14-18], others failed to show any beneficial effect of FAV [19-22].

In spite of scarcity of convincing and evidence-based data, our COVID-19 treatment strategy followed the in-hospital guidelines developed by a multi-disciplinary team based on updated guidelines issued by the Turkish Ministry of Health [23].

In this study, we compared the clinical outcomes and effects of the treatments on laboratory parameters between patients who were treated with FAV or HCQ/AZ.

MATERIALS AND METHODS

Study Design and Population

This single-center, retrospective study was conducted in Hacettepe University Hospital, a tertiary care hospital with 1200 beds for adult patients. We collected data of confirmed COVID-19 patients (older than 18 years old) hospitalized in COVID-19 wards between March 20, 2020, and September 30, 2020 retrospectively. Approval of the local ethical committee (Approval number: GO 20/353, date: 31.03.2020), and permission of the Ministry of Health of the Republic of Turkey were obtained.

The study enrolled all consecutive patients who met the following inclusion criteria: (a) patients 18 years or older age; (b) SARS-CoV-2 infection confirmed by polymerase chain reaction (PCR) test (c) hospitalized between March 20-September 30, 2020; (d) hospitalized at least five days in COVID-19 wards; (e) patients with pneumonia detected via CT ; (f) patients who did not require non-invasive/invasive mechanical ventilation (g) patients with "moderate" or "severe" disease according to according to the World Health Organization (WHO) classification (24) (h) patients who completed treatment as per protocol without early discontinuation due to any adverse reaction.

Critically-ill patients with sepsis and/or acute respiratory distress syndrome (ARDS) who required ICU care those with "mild disease" (without pneumonia) and "critical disease" according to the WHO classification (24) at the time of admission were excluded.

Clinical and laboratory data were retrieved from patient medical records until discharge, transfer

to the ICU or death in the ward. Decisions for hospitalization, treatment, transfer to the ICU and discharge were made by the Infectious Diseases consulting physicians and the primary consultants of the wards according to the hospital guidelines composed and regularly updated by a multi-disciplinary team of physicians based on the guidelines issued by the Scientific Board of the Ministry of Health of the Republic of Turkey [23].

Initially, patients with pneumonia received HCQ plus AZ (HCQ/AZ). FAV was not available in large quantities, and its use was restricted to critically ill patients who required intensive care unit (ICU) in the early days of the pandemic. Later, FAV became available widely and was preferred to treat patients with pneumonia regardless of the severity of the disease. Patients treated with HCQ/AZ received HCQ 400 mg twice on the first day, then 400 mg/day for 4 days plus AZ 500 mg on the first day, then 250 mg/day for 4 days. The standard protocol of FAV was 1600 mg of FAV b.i.d. on the first day, then 1200 mg/day (2x600 mg) for 4 days.

The discharge criteria in our center were absence of fever in the last 48 hours and clinical recovery, regardless of laboratory values.

Outcomes / Endpoints

The primary outcome of this study was to compare the changes in laboratory parameters in SARS Cov-2 infected patients treated with HCQ/AZ or FAV at admission and at discharge.

The secondary outcome was to evaluate the effect of the treatment in terms of transfer to the ICU, length of hospital stay, and/or exitus.

Propensity Score Matching

Since this was not a randomized trial, we used propensity score matching to estimate average treatment effect on laboratory parameters of COVID-19 infection in order to minimize the bias due to confounding factors, assuming that an imbalance in the patient background between the FAV and HCQ/AZ groups may exist.

The propensity score for each patient was calculated as a probability from a logistic regression model, including all important clinical and laboratory covariates that were shown to be of prognostic value [24]; a. gender, b. age, c. time from symptom onset to admission, d. symptoms such as sore throat, cough, myalgia - arthralgia, nausea - vomiting, diarrhea, loss of smell and/or taste, e. fever (body temperature ≥ 38° Celcius on admission), f. tachypnea (respirations ≥ 22/min), dyspnea, oxygen saturation (SpO2 ≥ 93% or lower) at admission g. comorbidities such as hypertension, diabetes mellitus, coronary heart disease, congestive heart failure, and/or chronic obstructive pulmonary disease, h. lymphocyte count, serum levels of ferritin, c-reactive protein (CRP), D-dimer and lactate dehydrogenase (LDH) on admission. In the propensity-score matching analysis, the nearest-neighbor method was applied to create a matched sample.

Statistical Analysis

Statistical analysis was performed with IBM SPSS for Windows version 23 package. The normality of numerical data was assessed with Shapiro Wilks test. Normally distributed continuous data were summarized by mean ± standard deviation, while non-normally distributed continuous data were summarized by median [25-75th percentiles]. The categorical variables were shown with the numbers and the percentages. The Chi-square test or Fisher exact test were applied to detect the relation between categorical variables. Independent sample t test or Mann Whitney U test was used to compare independent two groups in terms of numerical data. Within group differences were shown by Wilcoxon test. A 2-tailed p value of 0.05 was considered significant.

RESULTS

A total of 741 adult patients with laboratory confirmed COVID-19 were hospitalized in COVID-19 wards between March 20-September 30, 2020. Four hundred and eighty-one patients were excluded because of absence of pneumonia, hospital stay less than 5 days, early discontinuation of treatment due to adverse events or invasive/non-invasive mechanical ventilation (Figure 1). After propensity score matching, there were 42 patients who received FAV and 42 patients who received HCQ/AZ.

215 (82.7%) of 260 unmatched patients were treated with FAV and the rest 45 (17.3%) with HCQ/AZ. In this unmatched sample, there was a statistically significant difference in terms of age, hypertension,

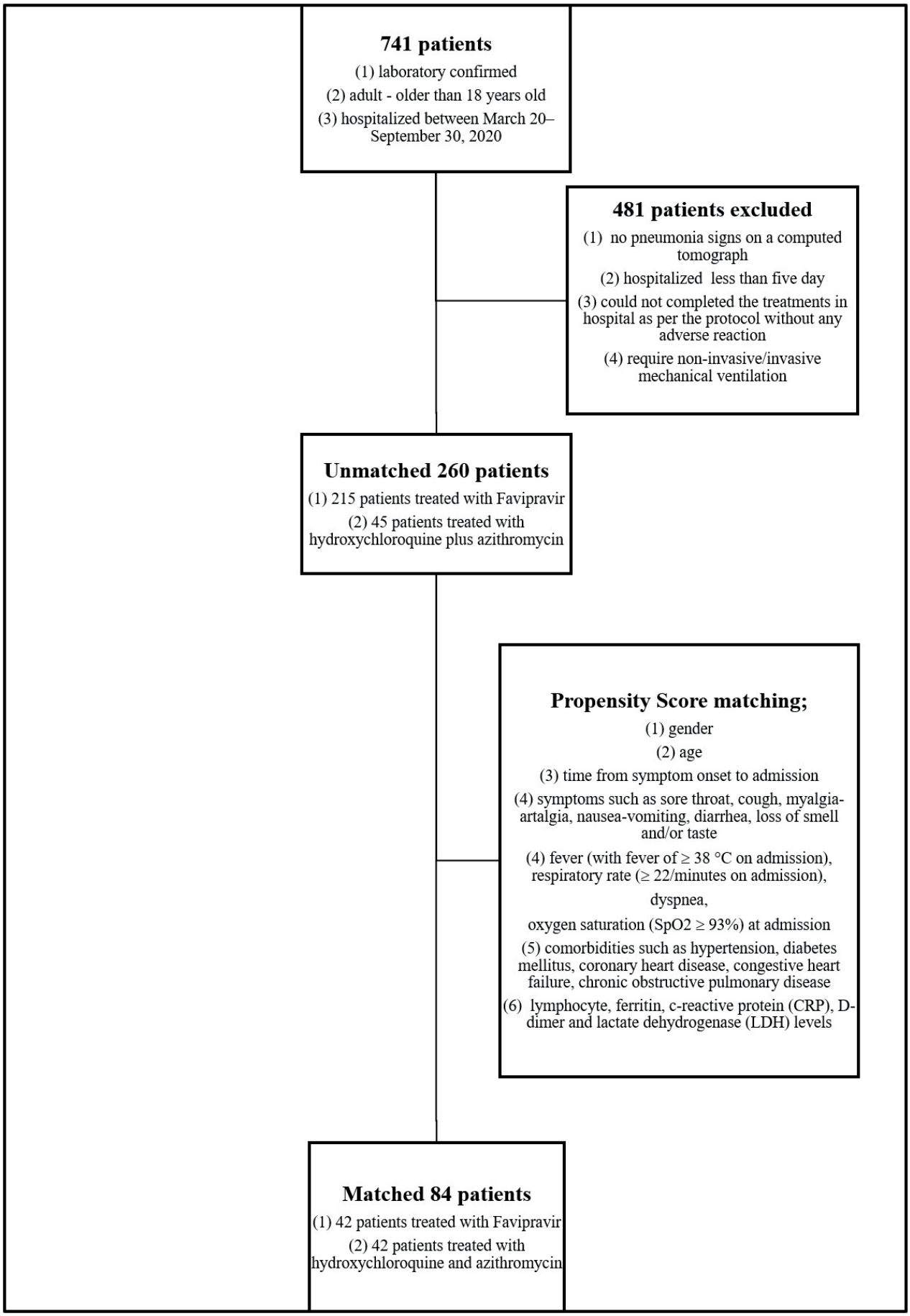

Figure 1. Case selection flowchart.

fever, respiratory rate, and oxygen saturation between treatment groups. The distribution of the baseline characteristics both in the unmatched and propensity-score matching analytic samples is shown in Table 1.

Propensity scores ranged from 0.00682 to 0.54438 in the FAV group, and from 0.01593 to 0.56627 in the HCQ/AZ group. While there were statistical differences between the patient groups in terms of transfer to ICU and/or exitus before matching (p=0.031), none of the treatment group was superior to the other in terms of discharge after propensity analysis (p=0.250). There were no statistically significant difference in terms of length of hospital stay between patients treated with FAV [9.02 days, SD: 6.4] and HCQ/AZ [8.14 days, SD:3.4] (p=0.903).

Total leukocyte counts increased at discharge in both treatment arms, but it was not significant. There was no difference between the two treatment groups in the measurements of leukocyte and neutrophil counts at admission and at discharge. On the other hand, the increase in lymphocyte and thrombocyte counts at discharge were statistically significant compared to admission values in both treatment arms. However, neither the increase in lymphocyte counts (p=0.956) nor platelet counts (p=0.280) were different between the two groups (See Table 2).

The levels of aspartate aminotransferase (AST), alanine transaminase (ALT), and LDH increased at discharge in both groups. The increases in AST (100.3% vs %39.4, p=0.043) and LDH (24.6% vs 9%, p=0.004) levels were observed more frequently in the FAV group compared to HCQ/AZ.

The levels of CK decreased significantly in the FAV group at discharge [167.6 (SD; 212) vs 110.12 (SD; 162.8), p=0.003]. Although there was a decrease in the HCQ group, it was not significant [118.2 (SD; 136) vs 87.7 (SD; 138.3), p=0.105]. Overall, there was no significant difference between the groups in terms of changes in CK levels at admission and discharge [34.3% vs 25.8%, p=0.071].

The changes in levels of CRP (p=0.167 at admission and p= 0.957 at discharge), procalcitonin (p=0.015 at admission and p= 0.121 at discharge),, and D-dimer (p=0.513 at admission and p= 0.383 at discharge), at admission and discharge were similar in any of the treatment arms. Both treatment groups were also comparable (See Table 2).

Finally, serum levels of ferritin and fibrinogen increased significantly during hospital stay in both groups whereas that of albumin decreased. The changes were similar in both treatment arms (See Table 2).

Although uric acid levels were mildly low in HCQ/AZ group at admission (5.13 vs 5.74 mg/dL), there were no statistical differences between the two treatment groups in terms of both uric acid levels at discharge (5.56 vs 5.01, p=0.164) and elevation of uric acid levels after treatments (p=0.399 for FAV group and p=0.427 for HCQ plus AZ group).

Only one (2.4%) patient in the HCQ/AZ group had nausea / vomiting whereas none who received FAV had any gastrointestinal discomfort. On the other hand, 9 (21.4%) patients in the FAV group and 4 (9.5%) patients in the HCQ/AZ group had more than 3-fold (but less than 5-fold) elevation in hepatic transaminases. There was no statistical difference between the groups (p = 0.227).

DISCUSSION

In this study, we showed that FAV was not superior to HCQ/AZ in terms of reducing transfer to ICU or exitus or the length of hospital stay, and although the levels of AST and LDH increased more frequently in the FAV group, both treatment regimens had similar effects in the values of laboratory tests at admission and discharge.

The efficacy of FAV in the treatment of COVID-19 is controversial. Early clinical studies with FAV from China showed reduction in viral load as well as improvement in clinical and radiological outcomes [15,25,26]. Two randomized trials failed to show that FAV was superior to CQ or HCQ. The efficacy of FAV was found to be similar to that of CQ for treatment of mild to moderate COVID-19 [27]. In the mentioned study, there were 48 patients in the CQ arm and 48 in the FAV arm. The length of hospital stay was shorter, and the need for mechanical ventilation was less among FAV-treated patients, but this did not reach a statistical significance. Our study also supports the similar efficacy of FAV to HCQ plus AZ for treatment or reducing transfer to ICU or exitus or the length of hospital stay in mild

Table 1. The distribution of the patients' baseline characteristics according to treatments both in the unmatched and propensity-score matching analytic samples.

	Unmatched, n=260			Matched, n=82		
	Favipravir n= 215	HCQ plus AZ n= 45	*P*	Favipravir n= 42	HCQ plus AZ n= 42	*P*
Age, mean (SD), year	59.32	46.69	**<0.001**	51.38 (17.152)	47.31 (15.203)	0.253
Sex, n (%)			0.820			1.0
Female	112 (52.1)	22 (48.9)		21 (50)	20 (47.6)	
Male	103 (47.9)	23 (51.1)		21 (50)	22 (52.4)	
Symptoms, n (%)						
Fever	131 (60.9)	27 (60)	1.0	25 (59.5)	25 (59.5)	1.0
Cough	115 (53.5)	30 (66.7)	0.146	23 (54.8)	29 (69)	0,261
Dyspnea	57 (26.5)	8 (17.8)	0.298	10 (23.8)	7 (16.7)	0.587
Myalgia	137 (63.7)	35 (77.8)	0.101	27 (64.3)	33 (78.6)	0,227
Nausea/Vomiting	23 (10.7)	9 (20)	0.139	5 (11.9)	7 (16.7)	0.755
Diarrhea	31 (14.4)	4 (8.9)	0.454	4 (9.5)	3 (7.1)	1.0
Headache	47 (21.9)	16 (35.6)	0.079	5 (11.9)	15 (35.7)	0.021
Sore Throat	31 (14.4)	15 (33.3)	**0.005**	6 (14,3)	14 (33.3)	0.073
Loss of Smell	14 (6.5)	5 (11.1)	0.340	1 (2.4)	4 (9.5)	0.360
Loss of Taste	12 (5.6)	2 (4.4)	1.0	1 (2.4)	2 (4.8)	1.0
Co-mobordities, n (%)				20 (47.6)	15 (35.7)	0.376
Diabetes mellitus	58 (27)	6 (13.3)	0.082	7 (16.7)	6 (14.3)	1.0
Hypertension	103 (47.9)	12 (26.7)	**0.015**	14 (33.3)	12 (28.6)	0.813
CAD	57 (26.5)	7 (15.6)	0.173	11 (26.2)	7 (16.7)	0.425
CHF	16 (7.4)	2 (4.4)	0.747	1 (2.4)	2 (4.8)	1.0
COPD	30 (14)	2 (4.4)	0.129	2 (4.8)	2 (4.8)	1.0
Malignancy	25 (11.6)	2 (4.4)	0.187	2 (4.8)	2 (4.8)	1.0
CKD	15 (7.0)	2 (4.4)	0.745	2 (4.8)	2 (4.8)	1.0
Immunsupressive treatment, n (%)	26 (12.1)	2 (4.4)	0.186	3 (7.1)	2 (4.8)	1.0
Admission						
Fever, mean (SD), °C	37.76 (1.02)	37.45 (0.96)	**0.047**	37.5 (1.07)	37.47 (0.93)	0.817
Fever, n (%) *						
< 38 C	90 (46.9)	29 (65.9)	**0.022**	17 (40.5)	17 (40.5)	1.0
> 38 C	102 (53.1)	15 (34.1)		25 (59.5)	25 (59.5)	
Respiratory rate, mean (SD)	20.8 (4.3)	19.48 (3.3)	**0.016**	20.61 (3.41)	19.6 (3.34)	0.098
Respiratory rate, n (%)						
< 22/min	131 (60.9)	38 (84.4)	**0.005**	33 (78.6)	35 (83.3)	0.781
> 22/min	84 (39.1)	7 (15.6)		9 (21.4)	7 (16.7)	
Saturation, mean (S.D)	93.8 (3.81)	95.81 (2.93)	**<0.001**	94.45 (3.26)	95.75 (2.98)	0.009
Oxygen Support, n (%)						
Not required	170 (79.1)	41 (91.1)	0.095	36 (85.7)	38 (90.5)	0.736
Nasal oxygen	45 (20.9)	4 (8.9)		6 (14.3)	4 (9.5)	
Disease Severity						
Moderate, n (%)	192 (89.3)	42 (93.3)	**0.587**	40 (95.2)	39 (92.9)	1.0
Severe, n (%)	23 (10.7)	3 (6.7)		2 (4.8)	3 (7.1)	
Outcome						
Length of Stay, mean (SD), days	9.93 (5.49)	7.96 (3.35)	**0.027**	9.02 (6.403)	8.14 (3.397)	0.903
ICU transfer, n (%)	17 (7.9)	0 (0.0)	**0.031**	1 (2.4)	0 (0.0)	0.250
Exitus	7 (3.3)	1 (2.2)		0 (0.0)	1 (2.4)	
Discharged	191 (88.8)	44 (97.8)		41 (97.6)	41 (97.6)	

HCQ; Hydoxychloroquine, AZ; Azithromycin, CAD: Coronary Artery Disease, CHF: Chronic Heart Failure, COPD: Chronic Obstructive Pulmonary Disease, CKD: Chronic Kidney Disease, °C: degree Celcius; *missing variables

Table 2. The comparison of Favipiravir with hydroxychloroquine + azithromycin therapies in terms of laboratory values alterations between the first (at admission) and the last day of hospitalization (discharge).

	Admission	*P*[1]	Discharge	*P*[2]	*Δ P*
Leukocyte (/mm^3), mean (SD)					
Favipiravir, n=42	5192.5 (2097)	0,830	5727.5 (2723)	0.593	0.361
HCQ plus AZ, n=42	5121 (1595.5)		5734.2 (1914)		0.112
Neutrophil (/mm^3), mean (SD)					
Favipiravir, n=42	3402.2 (1792.8)	0.731	3417.3 (2399.5)	0.217	0.397
HCQ plus AZ, n=42	3441.3 (1256.8)		3455.5 (1341.8)		0.766
Lymphocyte (/mm^3), mean (SD)					*P**= 0.956
Favipiravir, n=42	1202.3 (581)	0.564	1518.3 (794.2)	0.715	0.001
HCQ plus AZ, n=42	1193.7 (409.2)		2031.8 (2980.8)		0.001
Platelet (/mm^3), mean (SD)					*P**= 0.280
Favipiravir, n=42	195.5 (62.8)	0.132	241.8 (105.4)	0.497	0.002
HCQ plus AZ, n=42	172.5 (51.2)		254.6 (118.7)		0.000
Aspartate aminotransferase (AST) (U/L), mean (SD)					*P**= 0.043
Favipiravir, n=42	26.3 (9.3)	0.272	52.7 (47.5)	0. 454	0.000
HCQ plus AZ, n=42	30.7 (11.5)		42.8 (36.6)		0.009
Alanine aminotransferase (ALT) (U/L), mean (SD)					*P**= 0.070
Favipiravir, n=42	24.5 (19.7)	0.420	63.9 (60.3)	0.338	0.000
HCQ plus AZ, n=42	29.3 (16.8)		46.9 (42.1)		0.003
Lactate dehydrogenase (LDH) (U/L), mean (SD)					*P**= 0.004
Favipiravir, n=42	235.8 (106)	0.532	293,9 (104.1)	0.003	0.002
HCQ plus AZ, n=42	221.8 (103.5)		223.8 (96.9)		0.876
Creatin Kinaz (U/L), mean (SD)					*P**= 0.071
Favipiravir, n=42	167.6 (212)	0.613	110.12 (162.8)	0.992	0.003
HCQ plus AZ, n=42	118.2 (136)		87.7 (138.3)		0.105
C-reactive protein (mg/dL), mean (SD)					
Favipiravir, n=42	2.3 (2.1)	0.167	2.7 (2.9)	0.957	0.857
HCQ plus AZ, n=42	2.0 (2.3)		3.8 (5.9)		0.106
Procalcitonin (ng/mL), mean (SD)					*P**= 0.382
Favipiravir, n=42	0.63 (2.9)	0.015	0.13 (0.36)	0.121	0.400
HCQ plus AZ, n=42	0.06 (0.1)		0.05 (0.05)		0.932
D-dimer (mg/L), mean (SD)					
Favipiravir, n=42	0.77 (1.5)	0.513	0.71 (1.0)	0.383	0.851
HCQ plus AZ, n=42	0,75 (1.1)		0,75 (1.1)		0.681
Fibrinogen (mg/dL), mean (SD)					P*= 0.849
Favipiravir, n=42	380.2 (83.4)	0.934	448.8 (134.3)	0.860	0.006
HCQ plus AZ, n=42	351.3 (80.7)		435,4 (176,8)		0.021
Ferritin (µg/L) , mean (SD)					*P**= 0.096
Favipiravir, n=42	269.4 (554.3)	0.858	567.9 (992.1)	0.417	0.000
HCQ plus AZ, n=42	258.4 (628,8)		411.2 (903.6)		0.000
Creatinin (mg/dL), mean (SD)					*P**= 0.222
Favipiravir, n=42	0.95 (0.3)	0.141	0.84 (0.25)	0.264	0.002
HCQ plus AZ, n=42	0.83 (0.3)		0.78 (0.26)		0.024
Albumin (g/dL) , mean (SD)					*P**= 0.721
Favipiravir, n=42	3.97 (0.42)	0.323	3.60 (0.45)	0.400	0.000
HCQ plus AZ, n=42	3.94 (0.52)		3.59 (0.67)		0.000
Uric acid (mg/dL), mean (SD)					
Favipiravir, n=42	5.74 (1.61)	0.048	5.56 (2.03)	0.164	0.399
HCQ plus AZ, n=42	5.13 (1.90)		5.01 (1.62)		0.427

P[1] ; differences between parameters on admission, P[2]; differences between parameters at discharge, ΔP; differences between parameters (discharge - admission), P*; differences of alterations between groups

to moderate COVID-19 patients. Another recent study from Egypt compared FAV (50 patients) and HCQ plus oseltamivir (50 patients) in the treatment of mild and moderate COVID-19 cases [28], They concluded FAV was a safe effective alternative for HCQ in these patients. The average onset of SARS-CoV-2 PCR negativity was 8.1 and 8.3 days in HCQ-arm and FAV-arm, respectively; 55.1% of the patients on HCQ-arm became PCR-negative on/or before 7th day from diagnosis compared to 48% in FAV-arm (p=0.7). Four patients in FAV arm developed transient transaminitis whereas heartburn and nausea were reported in about 20 patients in HCQ-arm. Only one patient in HCQ-arm died after developing acute myocarditis that resulted in acute cardiac failure [28].

A recent meta-analysis did not reveal any significant difference between the intervention and the comparator on fatality rate (OR 1.11, 95% CI 0.64-1.94) and mechanical ventilation requirement (OR 0.50, 95% CI 0.13-1.95). There is no significant difference in fatality rate and mechanical ventilation requirement between FAV treatment and the standard of care in moderate and severe COVID-19 patients [29]. The results of our study support this meta-analysis and are valuable because of propensity matching.

The safety of FAV was evaluated in a review of 29 studies with a total of 4,299 participants and an estimated 175 person-years-of-follow-up [30]. There were significantly fewer gastrointestinal adverse events on FAV arm versus the comparators. The patients who received FAV showed significantly more uric acid elevations than those treated with comparators [5.8% vs 1.3%; P<0.0001]. Elevation in liver function tests were not observed. In our study, we could not detect any difference in terms of adverse effects (nausea/vomiting, liver enzymes elevation) between the two treatment groups. Only one (2.4%) patient in the HCQ/AZ group had nausea/vomiting.Doi et al. reported a total of 144 adverse events among 82 patients who received FAV; the most common was hyperuricemia (84%), followed by increases in serum triglyceride (11.0%) and serum ALT levels (8.5%) (20). In a prospective, observational study that included 174 hospitalized patients in COVID-19 wards, nausea, vomiting, and increase in transaminase levels were found to be higher in FAV group than those HCQ and AZ group [31]. In a study, pretreatment serum uric acid level has not found as a surrogate marker for the outcome of favipiravir treatment in COVID-19 patients, however, post-treatment uric acid levels were not observed in this study [32]. In our study, the pre-treatment levels were similar to mentioned study [32]; in addition, we did not observe a significant increase in uric acid levels after both FAV and HCQ/AZ treatments. In contrast, a study from Tokyo showed a high incidence of uric acid elevation, with regard to the established standards, in COVID-19 patients who received FAV therapy. The typical signs and symptoms such as gout and urinary stones were not observed in this study; however, uric acid levels increased more than 2.0-fold in 50% of these patients, and uricemia of moderate to severe intensity was recorded. In addition, the median onset time of uric acid elevation was 4.5 days [33]. The dosage of favipiravir and younger patient age were two potential risk factors for uric acid elevation. The dose of FAV in this study were higher than our study. Although the mentioned study [33] showed that uric acid levels may increase in high-dose FAV, it should be recommended to monitor uric acid levels closely in high risk patients who treated with FAV.

Inconsistent with Doi's study [20], there was no significant increase in ALT in the group using FAV, but a significant increase was observed in both AST and LDH with FAV than the HCQ/AZ treatment. However, it must be noted that patients who developed an adverse event that necessitated discontinuation of treatment were excluded in this study.

The primary outcome in the present study was the influence of FAV on some laboratory tests. During the course of COVID-19 infection, lymphopenia develops, the levels of some inflammatory parameters such as procalcitonin, CRP, ferritin and fibrinogen levels as well as hematological parameters such as D-dimer may increase [24] and these alterations have been reported to be of prognostic significance [24]. Even though improvement in these parameters could be expected with an effective drug such as FAV treatment, we could find no difference. On the contrary, a more significant decrease in d-dimer and CRP values in the group that received Favipiravir after HCQ before discharge compared to the group that received Favipiravir alone or HCQ alone was found in a study. However, the authors interpreted

the situation by the relationship between CRP and d-dimer reduction and disease recovery [34]. In our study the comparison between FAV and HCQ/AZ is insignificant in this regard. In the mentioned study [34], an evaluation was made on the 5th day independent of recovery and/or discharge. The comparison made when the patients met the discharge criteria could be more significant as in our study.

Our study has some limitations. As this was a retrospective study, we were unable to evaluate control imaging or time to PCR negativity as well as time to improvement in clinical parameters. The length of hospital stay was similar for both treatment arms (9.02 days vs 8.14 days, P=0.903), and all patients were afebrile for at least 48 hours and did not require supplemental oxygen at the time of discharge as per local guidelines. Although the number of patients seems to be low due to the study method, our study is valuable in that it presents real-life data.

In conclusion, FAV was not superior to HCQ/AZ in terms of reducing transfer to ICU or exitus or length of hospital stay. In addition, there were no differences in the change of laboratory parameters with a prognostic value in the course of COVID-19 infection between these two treatment modalities.

Author contribution

Study conception and design: ÖU, OAU, and NÇB; data collection: OAU, MÇS, and GTD; analysis and interpretation of results: OAU, MÇS, GTD, NÇB, SK, ÖU; draft manuscript preparation OAU, and ÖU. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Non-interventional Clinical Research Ethics Board (Protocol no. GO 20/353/31/03/2020).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

ORIGINAL ARTICLE

The Effect of Different Therapeutic Modalities on Demodex Densities and Clinical Symptoms of Patients with Demodicosis

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ABSTRACT

Background: Demodicosis represents cutaneous diseases caused by cutaneous overpopulation of Demodex mites. The aim of this study was to evaluate the effect of different treatment options on Demodex densities (Dds) and clinical symptoms of patients with demodicosis.

Methods: Patients with high Dds in two consecutive standardized skin surface biopsies (SSSB1>5 D/cm^2 or SSSB2>10 D/cm^2) and concomitant clinical symptoms were evaluated retrospectively. Measurements of treatment effectiveness included clinical improvement and normalization or reducing of Dds.

Results: A total of 21 patients included in the study. Five patients received topical permethrin and crotamiton whereas 16 patients received systemic metronidazole in combination with topical permethrin and/or crotamiton. The treatment was continued with topical ivermectin in 2 patients who had failure with other treatments. The median treatment duration was 3 months (IQR 1-4). Pre- and post-treatment median Dds decreased 30 to 14 D/cm^2 on SSSB1 whereas 81 to 80 D/cm^2 on SSSB2, respectively. There was no statistically significant decrease in Dds on SSSB1 and SSSB2 after the treatment (p=0.173 and p=0.134, respectively). Clinical improvement was recorded in a total of 14 patients (66.6%) of whom only 2 patients (9.5%) had normalization on Dds. Additionally, topical ivermectin provided a rapid clinical improvement and normalization on Dds in both 2 patients.

Conclusion: Irrespective of the treatment, more than two-thirds of the patients improved clinically without a significant change in Dds. This finding may suggest that the treatment response has been mostly associated with the anti-inflammatory properties of the agents. Topical ivermectin seems to be a more suitable treatment option for demodicosis with positive effects on both clinical findings and Dds.

Keywords: Demodicosis, demodex mites, treatment outcome

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INTRODUCTION

Demodex folliculorum is a microscopic mite which asymptomatically parasitizes the human pilosebaceous unit. The prevalence of mite increases with age up to 100% in late adulthood [1-3]. Although the role of *Demodex* mites as causative agents of human disease has been unclear, they are considered to play a pathogenic role when they multiply or penetrate to the dermis. The presence of more than 5 mites/cm^2 measured by the first standardized skin surface biopsy (SSSB1) or >10 mites/cm^2 on the second, deeper biopsy (SSSB2) defined as increased *Demodex* density (Dd) [1,4]. Increased numbers of mites have been identified mainly in demodicosis and papulopustular (PPR) or erythematotelengiectatic rosacea (ETR) [5-7].

Demodicosis is the term used to describe the cutaneous disease caused by increased *Demodex* mites and concomitant complaints including erythema, telangiectases, burning or stinging sensation, itching, scaling, dryness, irregular or rough skin [5,8,9]. "Pityriasis folliculorum" (PF), "rosacea-like demodicosis", and "granulomatous rosacea-like demodicosis" are the classical clinical forms of D. folliculorum infestation. Recently, rosacea-like demodicosis and PPR are considered to be the two phenotypes of the same disease [10]. It has been suggested to describe *Demodex* infestation in human beings in two clinical forms as noninflammatory demodicosis (NID) including PF and inflammatory demodicosis (ID) including rosacea-like demodicosis or PPR, demodex folliculitis, demodex pigmentation, follicular eczematids, isolated inflammatory papule [11]. NID manifests as a nutmeg grater appearance with discrete, fine, whitish, spiky follicular scales with or without faint erythema which can be completely asymptomatic or accompanied by dryness, itching, burning or stinging sensation [5,8,12,13]. ID usually shares the same features with NID and concomitant rosacea-like lesions consisting of papules and pustules [5,10,11,13]. The inflammatory stages can show predilection for perioral, periorbital and periauricular regions [12]. Less frequently, ID can manifest as folliculitis or abscesses, hyperpigmentation, follicular eczematids, isolated inflammatory papules, and ocular demodicosis.

Various treatments have been used for *Demodex*-associated skin eruptions, including topical sulfur products, permethrin, topical metronidazole, crotamiton, benzyl benzoate, ivermectin, tea tree oil (TTO), and systemic metronidazole or ivermectin [14-17]. However, there is no consensus on standard of care for the treatment of demodicosis yet. The aim of this study was to evaluate the effects of different treatment modalities on Dds and its impact on clinical outcomes in patients with demodicosis.

MATERIALS AND METHODS

We retrospectively analysed the data of patients who were diagnosed with facial inflammatory or non-inflammatory demodicosis in dermatology unit of a tertiary hospital in Turkey between January 2019 and December 2019. After obtaining ethical approval, data were collected from the electronic medical records of the patients. Demographic characteristics of the patients, clinical features with the type of demodicosis, type of treatment modality, clinical response to therapy, pre and post-treatment Dds, Dd normalization status were recorded. Patients with a history of immunosupression, pregnancy or lactation were excluded from the study. Informed consent was obtained for the diagnostic procedures from all patients.

The term "demodicosis" used for describing the patients who had increased Dd with any complaints of the followings: erythema, papules or pustules, burning or stinging sensation, itching, scaling, dryness, irregular or rough skin. Patients who had increased Dds with discrete, fine, whitish, spiky follicular scales with or without erythema but no papules or pustules accepted as NID. Patients who had increased Dds with central or periorificial papulopustules without comedones accepted as ID.

The patients were evaluated in two treatment groups, whether they received systemic treatment or not. Topical treatment group included topical permethrin once nightly in combination with crotamiton once daily whereas combined treatment

group included metronidazole tablet 500 mg two times a day in combination with topical permethrin and/or crotamiton. Dds (mite/cm^2) were measured by 2 consecutive SSSBs (superficial [SSSB1] and deep [SSSB2]) [4]. A density of more than 5 mite/cm^2 in SSSB1 or more than 10 mite/cm^2 in SSSB2 defined as positive result. Normalization defined as existing ≤5 mites/cm^2 in SSSB1 and ≤10 mite/cm^2 in SSSB2. The site used for SSSBs was the clinically affected zone, mainly the cheek of the patients if it was affected.

Measurements of treatment effectiveness included a decrease in Dd to normal levels (SSSB1≤5 D/cm^2 and SSSB2≤10 D/cm^2) and general reduction of Dds for each treatment. Secondary outcome measure was defined as clinical improvements in itching, burning or stinging sensation, erythema, xerosis, roughness, and papules or pustules of the skin. Clinical improvements were recorded as improvement, no improvement or worsening of the symptoms.

Statistical analysis

Statistical analyses were performed by SPSS software version 21.0 statistical package. Categorical variables summarized as frequencies and percentages. Descriptive analyses were presented using mean and standard deviation (SD) for the normally distributed variables or medians and interquartile range (IQR) for the non-normally distributed variables. Since the SSSB1 and SSSB2 measurements were not normally distributed; nonparametric tests were conducted to compare these parameters. Wilcoxon sign-rank test was used to compare the change in pre- and post-treatment Dds on SSSB1 and SSSB2. A p-value of less than 0.05 was considered to show a statistically significant result.

RESULTS

A total of 21 patients with demodicosis, 15 female and 6 male, were recruited in the study. The mean age was 43±13.02 years (range 21 to 66 years) and the median follow-up period was 4 months (IQR 1-4 months). The type of demodicosis were recorded as follows: 11 patients (52.3%) had NID and 10 patients (47.6%) had ID. Before the treatment Dds were positive on both SSSB1 and SSSB2 in all patients except 4 patients who had not been performed SSSB2.

Papulopustular lesions recorded in 10 patients with ID (47.6%). Erythema was seen in 18 patients (85.7 %) of whom 8 had PF and 10 had ID. Xerosis was noted in 11 patients (52.4%) of whom 7 had PF and 4 had ID. Fifteen patients (71.4%) had itching of whom 8 had PF and 7 had ID. Fourteen patients (66.7%) had roughness of whom 6 had PF and 8 had ID. Twelve patients (57.1%) had burning or stinging sensation of whom 6 had PF and 6 had ID. Three patients (14.3%) had hyperpigmentation.

There were 5 patients in topical treatment group and 16 patients in combined treatment group. The treatment was continued with topical ivermectin in 2 patients who had failure with topical or combined treatments. All 21 patients recommended to wash their face 2 times a day with soap or gel.

Pre and post-treatment Dds were summarized in Table 1 for each treatment groups. In topical treatment group, there was no statistically significant difference between pre and post-treatment Dds on both SSSB1 and SSSB2 (p=0.465 and p=0.686, respectively). SSSB1 and SSSB2 were positive in 4 patients (80%) whereas they were normalized in 1 patient (20%) after a median 4 months (IQR 2-4.5 months) treatment duration.

Table 1. Pre- and post-treatment Demodex densities of the treatment groups.

Treatment group	Pre-treatment Dds (D/cm^2) Median (IQR)		Post-treatment Dds (D/cm^2) Median (IQR)		P value	
	SSSB1	SSSB2	SSSB1	SSSB2	SSSB1	SSSB2
Topical	42 (13-60.5)	122 (44.5-199.5)	78 (6-105.5)	158 (9.5-190)	0.465	0.686
Combined	27.5 (17-53)	79.5 (39.8-151.3)	13 (4.8-41)	77 (17.8-94.5)	0.06	0.09
Overall	30 (17-50.5)	81 (41.5-150)	14 (5.5-43)	80 (17-106)	0.173	0.134

Dds: Demodex densities, SSSB: standardized skin surface biopsy

Clinical improvement was recorded in 4 patients (80%) of whom 1 patient (20%) had normalization on Dds.

In combined treatment group, there was no statistically significant difference between pre and post-treatment Dds on both SSSB1 and SSSB2 (p=0.06 and p=0.09, respectively). SSSB1 and SSSB2 were positive in 12 patients (75%), SSSB1 was negative and SSSB2 was positive in 3 patients (18.75%) whereas they were normalized in 1 patient (6.25%) after a median of 2 months (IQR 1-4 months) treatment duration. Metronidazole tablet was used with a median duration of 30 days, ranging from 5 to 90 days. Topical therapy was continued after the systemic metronidazole stopped. None of the patients with a systemic treatment reported any adverse effect or terminated the treatment early. Clinical improvement was noted in 10 patients (62.5%) of whom 1 patient (6.25%) had normalization of Dds.

The treatment was continued with topical ivermectin in 2 patients who did not responded the therapy. Of whom the first patient was recommended topical ivermectin after failure with 2 months usage of systemic metronidazole in combination with topical permetrine and crotamiton. The second patient was recommended topical ivermectin after failure with 4 months usage of topical permetrine and crotamiton. Before topical ivermectin therapy, Dds were 38 and 106 D/cm^2 for the first patient and 112 and 215 D/cm^2 for the second patient on SSSB1 and SSSB2, respectively. One month after treatment, Dds had normalized on SSSB1 and SSSB2 for both two patients. Clinical improvement was also noted on both of them.

Overall, there was no statistically significant difference between pre and post-treatment Dds on SSSB1 and SSSB2 in the whole group (p=0.173 and p=0.134, respectively). There were a 53.3% decrease on SSSB1 and 1.3% decrease on SSSB2 after the treatment. SSSB1 and SSSB2 were positive in 16 patients (76.2%), SSSB1 was negative and SSSB2 was positive in 3 patients (14.2%) whereas SSSB1 and SSSB2 were normalized in 2 patients (9.5%) after the treatment. With regard to clinical improvement in the whole group, 7 patients (33.3%) had no change whereas any clinical improvement was noted in 14 patients (66.6%) of whom 4 patients were in topical treatment group and 10 patients were in combined treatment group. No patients had worsening of the symptoms. When the improvement in each symptom after the treatment was evaluated separately; 60%, 33.3%, 36.3%, 53.3%, 50%, 66.6% and 66.6% improvement was recorded in papulopustular lesions, erythema, xerosis, itching, roughness, burning or stinging sensation and hyperpigmentation, respectively.

DISCUSSION

Demodex-associated skin diseases remain a diagnostic and therapeutic challenge in human beings. There are no standardized therapeutic recommendations for the treatment of human *Demodex*-associated skin diseases yet which may be mainly due to the lack of knowledge about the pathogenicity of the mite which also exists in healthy skin. Our study showed that irrespectively of the clinical features, demographics and treatment, our patients were clinically improved without a significant change in Dds. Additionally, both of 2 patients, whose treatment continued with topical ivermectin, had clinical improvement and normalization in Dds within 1 month.

In a recent systematic review about the treatment of *Demodex*-associated inflammatory skin conditions, topical permethrin is recommended as a first-line treatment option and oral metronidazole therapy as a second-line treatment option, with unknown long-term efficacy and safety [18]. In a recent larger study with 394 patients by Forton et. al. topical therapy with benzyl benzoate and crotamiton showed that Dds had normalized in 35% patients and symptoms had cleared in 31% of patient whereas both Dds normalized and symptoms had cleared in 20% of patients [16]. In current study, symptoms had improved in 80% of patients whereas Dds had normalized in only 20% of them after topical permethrin and crotamiton treatment. There was no significant decrease in Dds, conversely the median Dds increased on both SSSB1 and SSSB2 after the treatment. High clinical improvement rate with lack of significant decreasing in Dds may be related to the low acaricidal effect of these agents which cannot be demonstrated in a small sample in the current study.

SSSB is an easily accessible and practical tool in the determination of *Demodex* infestation. A second deep biopsy (SSSB2) performed at the same area

increases the sensitivity of the procedure by providing the sampling of deeper located mites [4]. Thus, if the post-treatment Dds were measured by only SSSB1 in our study, the normalization rate would have increased from 9.5% to 23.8%. We think that sampling with two consecutive SSSBs is important to provide more accurate and comparable results for future research.

There have been a few reports, mainly case reports, on systemic metronidazole therapy for *Demodex* mites with variable results [19-23]. In 2003 Schaller et al. reported a case of demodicosis treated with oral administration of 250 mg metronidazole three times a day for 2 weeks resulted in a rapid and long-lasting recovery including negative scrapings and symptoms following 9 months [21]. Hoekzema et al. reported complete clearing of symptoms and disappearance of facial mites in one patient, with oral metronidazole tablet (500 mg twice daily for 15 days) in combination with 1% metronidazole cream twice daily [23]. On the other hand, it was also reported that a patient who had only slight improvement with 750 mg of metronidazole for 8 months, cleared after 6 weeks of treatment with topical crotamiton [22]. A single-blind, randomized controlled trial by Salem et al. demonstrated that the combined therapy with two doses of ivermectin 200 mcg/kg orally given 1 week apart and metronidazole 250 mg orally three times daily for 2 weeks was superior in reducing the mite count to the normal level in rosacea and in blepharitis lesions from ivermectin alone [24]. They attributed the difference to the anti-inflammatory effect of metronidazole against the mite induced immune response. Supporting that idea, the clinical improvement rate of the symptoms (66.6%) were not associated with the normalization rate of Dds (9.5%) in our study. Although we cannot explain exactly whether the clinical improvement was caused by reducing Dds under a threshold level, even if not yet normalized, with a direct acaricidal effect or by anti-inflammatory properties of agents or both; the relatively high clinical improvement rate against the low normalization rate on Dds may suggest the anti-inflammatory effects of the agents are at the forefront. Since the mite has the ability for inducing inflammatory response, the effect of the agents in an anti-inflammatory way can be explained by suppression of mite-induced inflammation [19,25-27].

Topical ivermectin is a semi-synthetic, antiparasitic agent which is also approved by FDA for papulopustular rosacea in 2014 due to a dual mechanism of action, having both anti-inflammatory and acaricidal activity against *Demodex* mites [28-30]. Recently Trave et al. reported a complete remission of inflammatory lesions in 50 patients affected by PPR treated with topical ivermectin 1% once daily over 16 weeks. Thirty-two percent of their patients were positive for *Demodex* mites, and all of them reported to turn negative after 16 weeks [31]. Additionally, good responses to single dose or repeated weekly doses of oral ivermectin on ocular or cutaneous demodicosis have been reported before [15,32,33]. In accordance with the previous data, 2 patients who received topical ivermectin treatment in our study had both normalization of Dds and reducing in clinical symptoms within one month. Although the delayed benefit resulting from earlier treatments cannot be excluded, topical ivermectin can be considered as a preferential treatment option for *Demodex*-associated skin diseases due to both its acaricidal and anti-inflammatory effects.

The current terminology for describing human demodicosis is quite confusing. In general, human demodicosis has been classified into three main groups as PF, rosacea-like demodicidosis and granulomatous rosacea-like demodicosis. Chen W. and Plewig G. proposed a new classification that divides human demodicosis into a primary and secondary form [12]. According to this classification the primary demodicosis includes PF, papulopustular/nodulocystic or conglobate demodicosis, ocular and auricular demodicosis whereas the secondary form describes skin lesions associated with an abnormal increase of *Demodex* mites in patients with other known skin or systemic diseases including rosacea. Traditionally, rosacea-like demodicosis thought to differ from PPR in several clinical criteria; including its rapid onset, asymmetric distribution of more superficial and smaller papules or pustules with periorificial predilection, presence of follicular scales and pruritus without flushing or persistent erythema. However, it is not always straightforward to differentiate it from PPR with these clinical signs in daily practice. Moreover, some authors consider PPR and rosacea like demodicosis are two phenotypes of the same disease and proposed to describe

demodicosis in two clinical forms as NID and ID [11]. Indeed, NID may be considered to include PF or ETR with increased Dds while ID includes PPR with increased Dds or rosacea-like demodicosis. Additionally, vascular findings cannot help to clearly distinguish these two diseases [8,9,13]. In a study by Forton et al. 83% of the patients with PF reported to have vascular symptoms including persistent erythema or flushing [13]. In another study evaluating facial signs and symptoms of *Demodex* infestation showed 65.6% of the patients presented with nonspecific erythema and itching [9]. Similarly, erythema and itching were the most common findings in our study that observed in 85.7% and 71.4% of the patients, respectively. It was followed by roughness (66.7%), burning or stinging sensation (57.1%), xerosis (52.4%) and hyperpigmentation (14.3%). In the current study we preferred to describe patients with increased Dd and concomitant symptoms as NID and ID because we think that demodicosis may be an entity associated with another dermatosis, not secondary to it.

The limitations of this study include its retrospective nature and small sample size. Secondly, the heterogeneous treatment protocol and duration of follow-up period which makes it difficult to compare treatment responses.

Topical permethrin and crotamiton alone or in combination with oral metronidazole provided a clinical improvement in two-thirds of patients with demodicosis in the current study. It is interesting that irrespective of the clinical features, demographics and treatment, patients improved clinically without a significant change in Dds. The low normalization rate on Dds in patients with clinical improvement might suggest that the treatment response was mostly due to the anti-inflammatory properties of the agents. Although it needs to be confirmed in larger studies, topical ivermectin seems to be most effective and etiological option in demodicosis with positive effects on both clinical findings and Dds. Understanding the causative role of *Demodex* mites in the pathogenesis of human skin diseases will pave the way for more effective treatment options.

Author contribution

Study conception and design: BYA and NA; data collection: BYA; analysis and interpretation of results: BYA and NA; draft manuscript preparation BYA. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the local ethics committee (GO20/100/27.01.2020).

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

CASE REPORT

Dyspnea and Dysphagia as First Sign of Hypopharyngoesophageal Lipoma

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ABSTRACT

We present a rare case of a 72-years old male with a big lesion coming out from his mouth suddenly. Examination showed a mucosal lesion seen outside of the mouth, saliva running outside, he was not able to swallow, it was hard to speak and breathe for him. At the time of arrival the patient was stable, later developed a distress and dyspnoe because of the part of tumor inside of his mouth was moving in and out on breathing and was obstructing his airway. This situation required an urgent tracheostomy to secure the patient's airway. After tracheostomy we continued with endaural approach technique to remove the tumor, the origin of lesion was identified within the postcricoid area. Lesion was removed safely, sent to histology with no complication after. Results came back as a lipoma pedunculated. Literature search did not reveal any cases of limb lipoma presenting with a sudden hanging tumor causing airway compromise and dysphagia, all cases were diagnosed during routine examination. Our case has proven that hypopharyngoesophageal lipoma can present as an acute condition and we have to be able to save and manage patients like this.

Keywords: Lipoma, limb lipoma, pedunculated lipoma, acute distress syndrome, dysphagia

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INTRODUCTION

Gastrointestinal tract inflammatory fibroid polyp is an uncommon, benign, submucosal lesion. Ward "lipoma" first was mentioned by Oslon in 1709. The „gastrointestinal tract lipoma '' was described in 1949 by Vanek. „Oral lipomas" were used by Roux in 1884, he described it as a „yellow tumor" [1,2]. Lipoma is a benign tumor derived from adipose tissue. It is a common mesenchymal tumor typically coming from the fat tissue, most commonly from the subcutaneous tissue. Microscopically: well-defined, encapsulated floating, on touch brittle tumor. Tumor can have a lobulated shape, which is then appears huge. Lipoma can be classified as superficial or deep. Variation of the superficial lipoma - „lipoma pendulum" - a floating, polypoid tumor typical for the digestive tract and skin [3,4].

Benign tumors of the esophagus are very rare, about 0.5-0.8 % of all esophageal neoplasms. Approximately, 60 % of benign esophageal neoplasms are leiomyomas, 20% are cysts, 5% are polyps, and less than 1 % are lipomas [5]. The upper third of the esophagus is the most common site of origin for digestive tract lipoma. Men present with this lesion more frequently than females. Lipoma can be a random finding on imaging or endoscopic examination. More than 85 % are asymptomatic [3]. Huge size lipoma can cause airway obstruction, dysphagia, foreign body sensation, regurgitation and even pain [6]. Endoscopic techniques, images - CT, MRI can be used for diagnosis. Gastroesophageal examination may be complicated by insufficient esophageal lumen, which can be partially or completely filled with tumorous mass [7]. On CT, the

lipoma appears as a homogeneous hypodense well-defined mass. CT is beneficial for lipoma diagnosis, but for soft tissue structures MR imaging is a gold standard [8]. Indication for surgical intervention is: symptomatic patient, suspicious of malignancy or possible transformation, but it is very rare [5,7].

CASE PRESENTATION

During on-call duties, we had a phone call from ENT colleagues from another ENT department about 72- years old patient with a tumor hanging out from his mouth, this patient has an intermittent stridor. An urgent CT was performed and showed a pedunculated polypoid mass size about 41x33 mm, 22x14 mm out of mouth, other part of mass within the oral cavity about 30x20 mm. Mass origin is likely from the oro-hypopharyngeal wall, or esophageal wall. Esophageal lumen was dilated, the epiglottis was depressed by mass anteriorly partially obstructing glotis (Figures 1, 2 and 3).

Patient arrived within an hour by ambulance, was asymptomatic, stable. Patient has a history of not being well last night, starting to cough, to drool, to vomit. We suppose that during vomiting mass has come out from his mouth and he got worse with his breathing. On examination a polypoid mass was hanging out from mouth (Figure 4), saturation started to drop, sequently, patient transferred to the intensive unit care to control his breathing. Patient

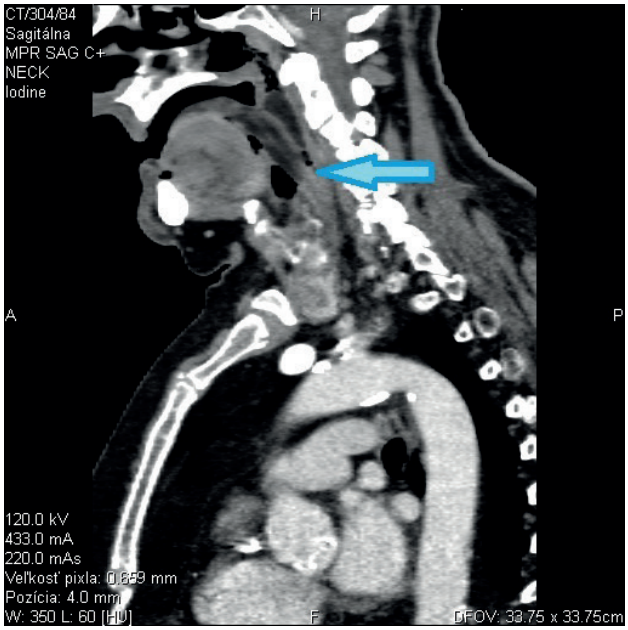

Figure 1. Sagittal CT scan of the tumor (marked by arrow).

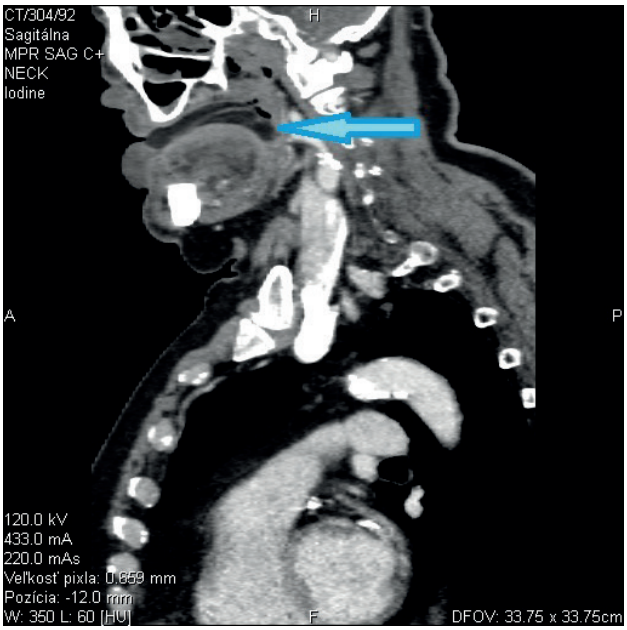

Figure 2. Sagittal CT scan of the tumor (marked by arrow).

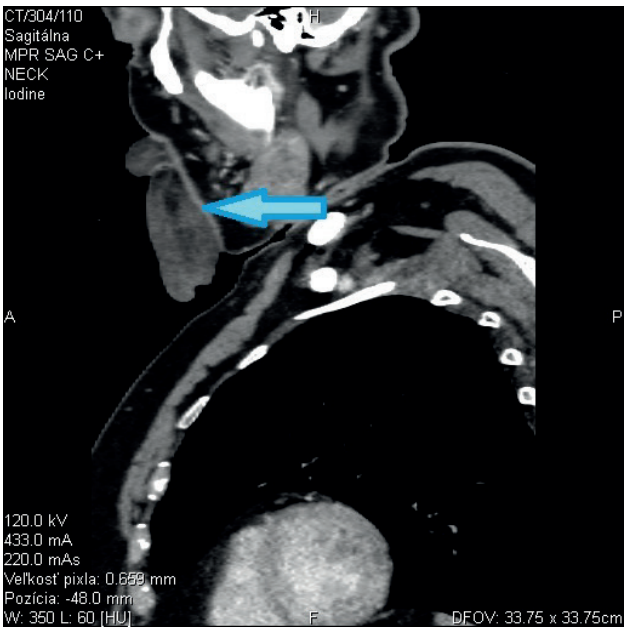

Figure 3. Sagittal CT scan of the tumor (marked by arrow).

became symptomatic, breathless, saturation went down. The floating mass in the oral cavity was seen, lesion was pulled out from the mouth manually. Patient was transported to operation room still holding the mass manually, then an urgent tracheotomy was performed.

Following it, under general anesthesia we identified a huge lobular tumor with origin from the mucosal surface of the posterior wall of the hypopharynx postcricoid area, (Figure 5). It completely fills the oral cavity, and then the tumor was removed safely

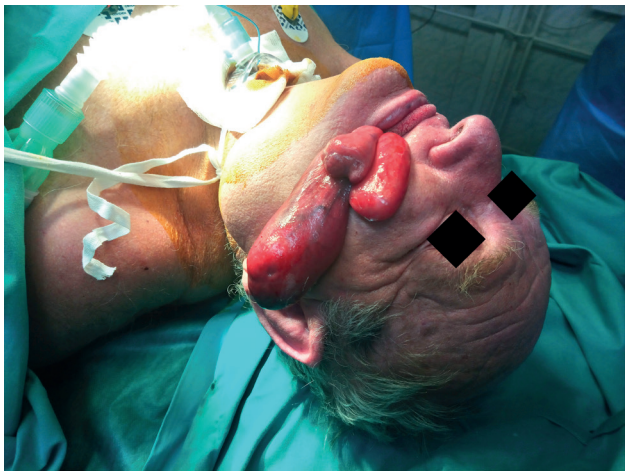

Figure 4. Patient with polypoid mass hanging out from mouth (after tracheostomy).

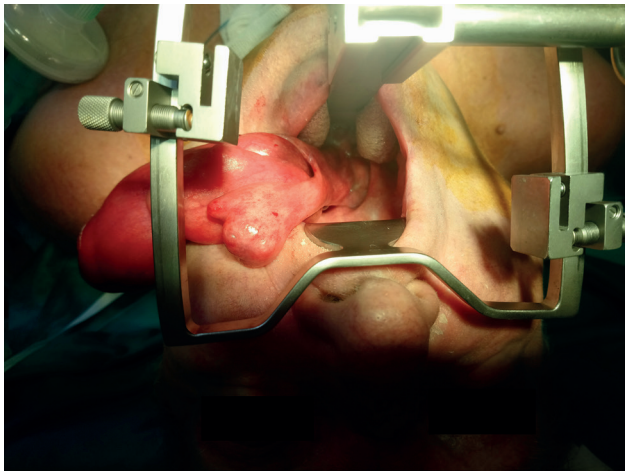

Figure 5. Intraoral view helping by Kaspenbauer´s spreader.

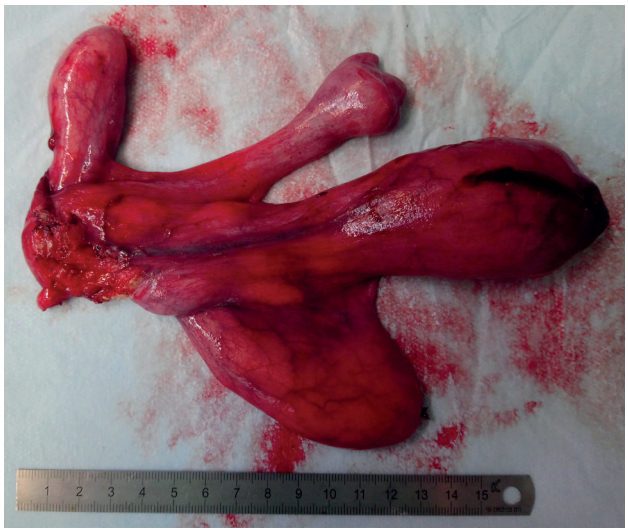

Figure 6. Tumor after removal.

(Figure 6). Nasogastric tube was inserted. Tumor was sent for histological examination, result was - lipoma pendulum, limb lipoma.

After the patient has recovered from the procedure, we could get more history from the patient's relatives. Patient has had a family celebration. He was in a good condition before, without any serious problem with digestive tract, no vomiting, no coughing, with no previous gastrointestinal problems or examinations. Tumor comes up suddenly without any warning signs. He also suffered from high blood pressure, ischemic heart disease, AV block, benign prostatic hyperplasia.

No complication was seen during hospitalisation, the patient was decannulated on the 5th postoperative day, nasogastric tube was removed on the 9th postoperative day. Patient safely discharged home. Follow up after two weeks, patient remain asymptomatic, on endoscopic examination tumor free.

DISCUSSION

Lipomas have been found in all parts of the digestive tract, most often in the intestine. Esophageal lipoma is a very rare tumor, asymptomatic, mainly identified accidentally, it has never been described as an acute condition or as a life- threatening. In literature we haven´t found lipoma coming out of mouth as a first sign in diagnostics.

In the differential diagnosis in the esophagus, we mainly think of Zenker's diverticulum. As far as the fatty tissue tumor, we start to think about liposarcoma, which is one of the most common sarcomas in adulthood. Well-differentiated liposarcomas are clinically low-malignant only rarely metastatic sarcomas, with less-to-poorly differentiated liposarcomas often being high-malignant sarcomas. There are several histological subtypes of lipomas such as simple lipoma, fibrolipoma, mixed lipoma, chondroid lipoma, angiolipoma, angiomyolipoma, myelolipoma, spindle lipoma, sialolipoma, pleomorphic lipoma and atypical lipoma [9]. In a differential diagnosis in dyspneic disorders, it may be a lipoma that grows directly from the larynx, which is also rare; only a few cases are described in the literature [10,11].

Mayo et al. present 4,000 clinical cases of benign neoplasia. From these benign neoplasia, lipomas formed 4.1 % , esophageal lipomas 0.4 %. Nora presents 17 oesophageal lipomas, of which 16 were

located intraluminal. Akiyama et al. documented 10 esophageal lipomas, 7 of which were in the cervical and 3 in the thoracic region. Moerscha and Harrington study included 7,459 drinks, where 44 benign esophageal tumors were randomly found and in only two cases it was a shy lipoma [5,12].

The choice of surgical technique depends on the localization, size and accessibility of the tumor. Endoscopic techniques are more often popular, as it shortens length of hospitalisation and recovery of the patients. It is associated with a lower risk of complications (such as, pulmonary atelectasis, dystelectasis and pleural effusion) [12]. Open surgical techniques are used for better clarity and easier ligation of blood vessels. It is beneficial for the impossibility of removing enormous mass through the esophageal lumen. The use of a transoral, transthoracic, or transcervical approach depends on the size of the tumor, the location of the intraluminal mass and the origin of tumor /from the hypopharyngeal or esophageal wall/, the risk of airway injuries and large vessels [7,12].

In the literature, the tumor of the upper third of the esophagus is presented through a right-handed mini-thoracotomy with a video-assisted thoracoscopic technique [5]. Weigel et al. present a case of resection of the giant stemmed lipoma of the lower third of the esophagus through the transgastric laparoscopic approach [12]. In our case a transoral approach was used. Tumor´s origin was identified on the low part of the posterior pharyngeal wall near the postcricoid area; the tumor was removed safely with no complications.

Gastrointestinal tract lipomas are rare, but esophageal lipomas even more. They represent 0.03 % of all esophageal benign neoplasia. Presentation is often asymptomatic, however, sometimes it can be associated with breathing difficulties and can lead to apnoea. In our case, lipoma was huge. The patient suddenly became breathless and went into acute distress syndrome. We would like to emphasize that lipoma coming out of the oral cavity can be a life- threatening. We have to be aware about this rare tumor, we need to be ready to face it if a situation like this comes to our practise.

Author contribution

All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

It is not necessary to case report.

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Conflict of interest

The authors declare that there is no conflict of interest.

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acta medica

CASE REPORT

Anesthetic Management with Dexmedetomidine During the Awake Craniotomy Surgery: A Case Report

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ABSTRACT

Objective: Awake craniotomy is a procedure of choice when the area to be resected is close to the eloquent regions of the cerebral cortex. In this case report, the anesthetic technique in the awake craniotomy case presented.

Case Presentation: A 62 years old female was scheduled for awake craniotomy procedure. Anatomically, the tumor involved the motor tracts of the right hand. Following the intravenous access and monitorization of the patient, 4.5 mg midazolam and 50 mcg of fentanyl used for sedation of the patient and dexmedetomidine was used for maintenance. We used Bispectral Electroencephalogram Index in order to measure the deepness of sedation during the surgery. Desaturation and hypercapnia on the arterial blood gas analysis seen following the addition of 3 mg of midazolam to deeper the sedation after hemostasis. The dose of dexmedetomidine increased and flumazenil administered to reverse the midazolam.

Discussion: Dexmedetomidine belongs to alpha-2 adrenergic agonist group, which acts through its sedative effects at the locus coeruleus. Different from GABA, alpha-2 adrenoreceptors produce sedation without the entire spectrum of stupor letting patients stay somnolent and easily awakened with verbal stimuli becoming compatible to be tested. In conclusion, dexmedetomidine is one of the possible choices of medication for awake craniotomy as it maintains the patient's convenience, reduces analgesic needs, and level of deliberateness without any confusion and agitation.

Keywords: Awake craniotomy, conscious sedation, dexmedetomidine, midazolam

INTRODUCTION

Awake Craniotomy procedure requiring verbal communication with the patient during the procedure demands a unique approach of the anesthesiologist and this is the procedure of choice when the area to be resected is close to the eloquent regions of the cerebral cortex.

Dexmedetomidine is the medication of choice with its pharmacokinetic properties like rapid distribution half-life being approximately 5-6 min and an elimination half-life of approximately 2 hours [1,2]. The first case report introducing the successful use of dexmedetomidine for awake craniotomy was performed by Bekker et al. in 2001 [3]. Later on, Souter et al. [4] published several cases indicating the successful use of dexmedetomidine and its combination with fentanyl. In this paper, we reported the anesthetic technique in the awake craniotomy case where dexmedetomidine hydrochloride was used in combination with fentanyl and midazolam for the procedure and cortical mapping.

CASE REPORT

A 62 years old, 56 kg female with right parietal lobe glial tumor was scheduled for awake craniotomy procedure. On the preoperative assessment of patient besides hypertension regulated with valsartan + hydrochlorothiazide and the history of cholecystectomy operation under general anesthesia, nothing remarkable was found. Her physical status was evaluated as ASA (American Society of Anesthesiologists) II. Anatomically, the tumor involved the motor tracts of the right hand and on physical examination of the patient right-hand weakness and writing difficulty was detected. During the procedure as well as patient's hand movement and motor functions were also controlled.

No premedication was administered to the patient. After the acceptance to the operating room, the patient was monitored with a pulse oximeter, electrocardiogram, radial arterial line for invasive blood pressure and axial skin temperature. The deepness of sedation was measured with bispectral electroencephalogram index (BIS) monitor. EMG monitorization was also performed for the assurance of cranial nerves. Foley catheter was inserted and urine output was monitored. The patient was positioned in the supine position. Following the patient's acceptance to the operating room and the intravenous access with 20G and 18G intracaths, 4.5 mg midazolam and 50 mcg of fentanyl used for a sedation of the patient. Dexmedetomidine initial loading dose of 1 mcg/kg was administered for 10 minutes and then 0.3 mcg/kg/hour as the maintenance dose of infusion. The scalp nerves were blocked bilaterally with 60 mg 0.5% bupivacaine and 240mg 2% prilocaine. Desaturation and hypercapnia on arterial blood gas analysis (SpO_2 = 88% and $PaCO_2$ = 47mmHg) occurred when the sedation level deepered with IV injection of 3 mg of midazolam following the hemostasis. For reversing the effects of midazolam and not to use a laryngeal mask airway (LMA), 0,5 mg flumazenil injected intravenously and then sedation level deepered by increasing the infusion dose of dexmedetomidine. During the operation, the BIS index varied between 70 and 90. We observed heart rate about 60-70 bpm, optimum blood pressure levels and body temperature between 36.0 to 36.5 °C during the procedure. For this report, writen informed consent was obtained from the patient.

DISCUSSION

There are different anesthesia techniques from local to general used for language mapping and tumor resection during an awake craniotomy. Dexmedetomidine represents a different criterion in anesthesia. Dexmedetomidine belongs to alpha-2 adrenergic agonist group which acts through its sedative effects at the locus coeruleus. Different from GABA, alpha-2 adrenoreceptors produce sedation without the entire spectrum of stupor letting patients stay somnolent and easily awakened with verbal stimuli becoming compatible to be tested [5].

The common inconvenience when dealing with awake surgery is anxiety. Due to the reduction of sympathetic responses to stress at the central and peripheral nervous system 0,2-0,7 mcg/kg/hour dexmedetomidine has a strong anxiolytic effect. Undeniably, we see that patients on this drug were less anxious than expected. The dose of 0.3 mcg/kg/hour dexmedetomidine, in this case, produced an optimal somnolence. In contrast to other studies, we had neither significant hemodynamic instability nor bradycardia in our case.

During an awake craniotomy procedure, pain control is one of the most important tasks. While retracting the temporal muscle and deattaching of cranial bone from dura mater near the temporal fossa, patients feel pain, which requires an increase in analgesia. Anesthetic rearrangement may cause drowsiness and as right after the craniotomy, brain mapping should be done, before the verbal testing patient may need some time for recovery which may affect the total time of surgery. However, using dexmedetomidine as the main agent decreases the need for other analgesic drugs enabling neuropsychological assessment comfortable. In our case study, we used totally 100 mcg of Fentanyl as an analgesic agent.

In our case, we did not monitor any significant oxygen desaturation or hypercapnia except the second bolus dose of 3 mg midazolam in accordance with the literature. The brain kept appropriate for microsurgery by controlling fluid balance and sufficient spontaneous ventilation with no need to use a laryngeal mask airway.

During the awake craniotomy procedure, the risk of convulsions increases leading anesthetists to be

prepared for the treatment of convulsive incidents. Although there is no evidence describing human cases, it has been proved that dexmedetomidine decreases the convulsion threshold in animal models (6). We did not face any seizures in our case.

CONCLUSION

In conclusion, dexmedetomidine is one of the possible choices of medication for awake craniotomy as it maintains patient's convenience, reduces analgesic needs, and level of deliberateness without any confusion and agitation. Although the usage of dexmedetomidine as a sole source causes no significant respiratory depression and does not require LMA, but it should be taken into account that in combination with midazolam it may lead to respiratory depression as well as saturation decrease.

Author contribution

Study conception and design: SU, Mİ and TR; data collection: Mİ and TR; analysis and interpretation of results: Mİ and TR; draft manuscript preparation SU, Mİ and TR. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Ethical approval is not required.

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Conflict of interest

The authors declare that there is no conflict of interest.

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