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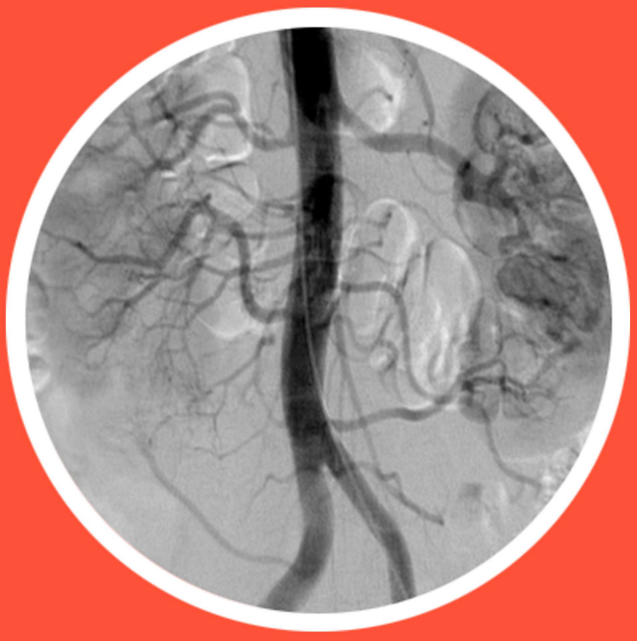

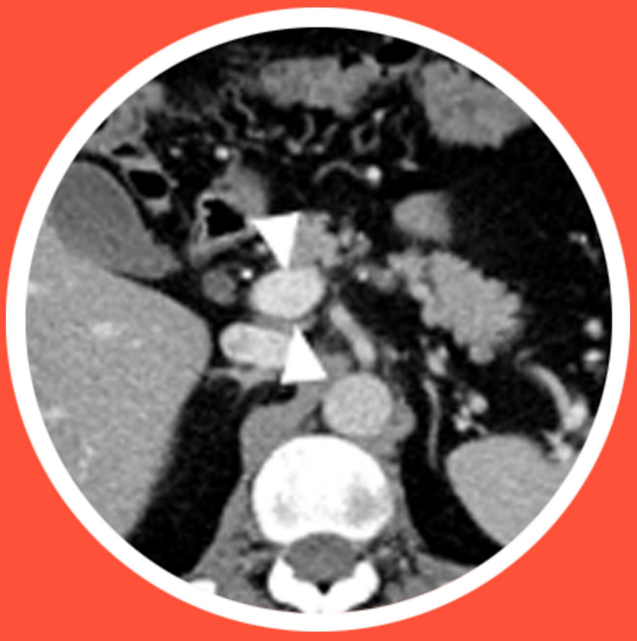

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Original Articles

Case Report

The European Research Journal 2021;7(5):440-446 Original Article

DOI: 10.18621/eurj.679456 *Neurology*

Relevant muscle selection for needle electromyography examination in polio survivors

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ABSTRACT

Objectives: Needle electromyography (EMG) has an important role in the diagnosis of poliomyelitis. Since needle EMG is a painful and time consuming procedure, selecting the most effective muscles for needle EMG is important. We aimed to determine the clinically or subclinically affected regions in polio survivors by examining the minimum number of muscles with EMG.

Methods: Polio survivors with weakness in at least one limb were included in this retrospective cohort study. The extremities were divided into two groups: 1) Group 1: The extremities with Medical Research Council score of ≤ 1 in at least one muscle, 2) Group 2: Clinically unaffected or mildly to moderately affected extremities that do not meet the criteria of Group 1. The needle EMG findings of the muscles were analyzed.

Results: Twenty-nine polio survivors were included in the study. Needle EMG findings of 352 muscles were reviewed. Needle EMG findings in 57 lower extremities and thirty-two upper extremities were analyzed. There was no upper extremity meeting the criteria of Group 1. Thirty-eight lower extremities were included in Group 1. The amplitudes and durations of motor unit action potentials (MUAPs) were not different between the muscles of the Group 1 lower extremities ($p > 0.05$). Among the muscles of Group 2 upper and lower extremities, the amplitude and duration of MUAPs were higher in the deltoid and the vastus lateralis muscles compared to the other muscles, respectively ($p = 0.002$ and $p = 0.003$ for upper extremity muscles; $p = 0.005$ and $p < 0.001$ for lower extremity muscles).

Conclusions: Using the needle EMG findings, an algorithm was made to determine the affected regions. Thus, the affected regions can be identified by applying needle EMG to a minimum number of muscles.

Keywords: electrodiagnosis, needle electromyography, poliomyelitis, polio survivors

Poliomyelitis is a disease caused by RNA enterovirus affecting the brain and the motor neurons of the spinal cord and brainstem. Poliovirus caused many deaths and disabilities in the 1940s and 1950s; fortunately, it is rarely seen today with the development of vaccines [1, 2]. On the other hand, patients with polio sequelae continue to have health problems such as musculoskeletal problems or late neuromuscular deterioration. Poliovirus affects most motor neurons and destroys about 50% of these motor neurons [1, 3, 4]. After this acute phase, the disease may remain stable over the years. In some patients, late neuromuscular deterioration or post-polio syndrome may develop in addition to the polio sequelae

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after years.

The cause of the neuromuscular deterioration is unknown, but it is thought that factors such as excessive exercise, chronic poliovirus infection, changes in muscle fiber metabolism, or immune-mediated mechanisms may be triggers [1, 5, 6]. Regardless of the cause, motor neurons are damaged faster than normal aging [7, 8].

It may be important to demonstrate the clinical and subclinical involvement with needle electromyography (EMG) [9]. Thus, protection of motor neurons or axons can be provided by physical therapy strategies. Needle EMG is a painful and time-consuming electrodiagnostic test, so it is not possible to evaluate all the muscles of a polio survivor with needle EMG. Therefore, it was aimed to select the most effective muscles for needle EMG examination and find the clinically / subclinically affected extremities in polio survivors by examining the minimum number of muscles with needle EMG.

METHODS

Subjects

Patients with polio sequelae admitted to EMG laboratory between July 2018 and December 2019 were included in this retrospective cohort study. Ethics committee approval was received from the ethics committee of Adana City Training and Research Hospital (number: 46/636). The clinical features and electrodiagnostic findings of patients with a history of poliomyelitis and weakness in at least one limb were reviewed. The patients who had polyneuropathy or a disease that could cause polyneuropathies such as diabetes mellitus and those with a history of surgery due to lumbosacral and cervical radiculopathy or findings suggesting significant cervical or lumbosacral radiculopathy in magnetic resonance imaging or computer tomography were excluded from the study. Medical research council (MRC) scale was used for muscle strength assessment [10, 11]. The MRC scores of arm abduction, elbow flexion, elbow extension, wrist extension, and finger abduction were analyzed in the upper extremities. MRC scores of hip flexion, knee flexion, knee extension, ankle flexion, and ankle dorsiflexion were analyzed in the lower extremities. The muscles with an MRC score of 0 and 1 cannot resist a force [10, 11]. For this reason, it is difficult to obtain the motor unit action potential (MUAP) with needle EMG in muscles with MRC scores ≤ 1. Therefore, the extremities were divided into two groups: 1) Group 1 was defined as clinically severely affected extremities with an MRC score of ≤ 1 in at least one muscle examined, 2) Clinically unaffected or mildly to moderately affected extremities that did not meet the criteria of Group 1 were defined as Group 2.

Electrodiagnostic Tests

The nerve conduction studies and the needle EMG were performed with the Cadwell Sierra Summit EMG unit (Cadwell Laboratories, Kennewick, Washington, USA). Electrodiagnostic tests were performed when the extremity temperature was ≥ 32 ºC; otherwise, the extremities were heated. Surface electrodes were used for stimulation and recording. Median, ulnar, posterior tibial, peroneal, sural sensory, and motor nerve conduction studies were performed using conventional methods. Band-pass filters for sensory and motor nerve conduction studies were set at 20 Hz to 2 kHz and 20 Hz to 10 kHz, respectively. For the reference values of nerve conduction studies, the reference values recommended by the American Association of Neuromuscular & Electrodiagnostic Medicine were considered [12]. Needle EMG was performed with a concentric needle electrode (length = 50mm, diameter = 0.46mm, Bionen Medical Devices, Florence, Italy). Low and high-frequency filters for needle EMG were 10 Hz and 10 kHz, respectively. For the analysis of spontaneous activity, sensitivity and sweep speed were set to 100 uV/division and 10 ms/division, respectively. The low and high filters for the analysis of the MUAPs were 200-1000 uV/division and 10 ms/division, respectively. In polio survivors with lower limb weakness, needle EMG was applied to the muscles of both lower extremities and the muscles of one upper extremity. In addition, if upper extremity weakness was present, needle EMG was applied to the muscles of both upper extremities. Although the trapezius muscle is innervated by both the 11th cranial nerve and the cervical segments, needle EMG was applied to the trapezius muscle to determine cranial nerve involvement. Needle EMG was performed visually. Positive sharp waves (PSWs) and fibrillation potentials (FP)

and fasciculation potentials were carefully examined. The severity of PSWs and FPs were evaluated as follows [13]: (0) Absence of PSWs and FPs, (1) Single PSW or FP in at least two areas, (2) A moderate number of PSWs or FPs in three or four areas, (3) PSWs or FPs in all areas and (4) PSWs or FPs filling the screen in all areas. During mild muscle contraction, at least 10 MUAPs were analyzed. If the amplitude of the MUAPs were > 4 mV or the duration of the MUAPs was > 15 ms, the MUAPs were considered neurogenic. At least 10 MUAPs were attempted to be analyzed from one muscle, but if the MUAPs were few, these MUAPs were taken into account. Among the MUAPs obtained from a muscle, those with the highest amplitude and longest duration were analyzed. In the case of neurogenic needle EMG findings in two muscles innervated by different segments and different nerves, this region was considered to be affected.

Statistical Analysis

The Shapiro-Wilk test was used to determine the distribution of the data. Comparisons were made using the Friedman test and Wilcoxon signed-rank test for dependent samples. Bonferroni correction was used as post hoc analysis. Pearson's Chi-squared test was used to analyze categorical variables. Mean ± standard deviation (SD) and median of numeric data were calculated for descriptive statistics. Statistical Package for the Social Sciences (SPSS IBM Corp; Armonk, NY, USA) 22.0 was used to perform the statistical analysis.

RESULTS

Thirty-two patients with polio sequelae were evaluated. Two patients with diabetes mellitus and one with a history of surgery due to lumbosacral radiculopathy were excluded, so twenty-nine polio survivors were included in the study. The clinical findings of seven patients were compatible with post-polio syndrome. Clinical findings and nerve conduction studies of two patients were consistent with carpal tunnel syndrome, and the needle EMG findings of the abductor pollicis brevis muscles of these patients were excluded. Nineteen patients (66%) were male. The mean age of the patients was 45.6 ± 7.5 (range 31-59) years. The mean age of the patients with acute poliomyelitis was 2.0 ± 1.2 (range 1-5) years. There was no patient with nerve conduction finding consistent with polyneuropathy. Fourteen patients had weakness in only one lower extremity, while 12 patients had weakness in both lower extremities. Ten of 12 patients with bilateral lower limb weakness had an asymmetric weakness. There were two patients with weakness in one upper and bilateral lower extremities and one patient with weakness in the left upper and right lower extremities. The compound muscle action potential (CMAP) of 56 posterior tibial nerves and 54 peroneal nerves were evaluated. The CMAP amplitude was reduced in 13 (23%) of 56 posterior tibial nerves and not obtained in 14 (26%) of 54 peroneal nerves. Seventeen (59%) of 29 patients had reduced CMAP amplitude or

Table 1. Needle EMG findings in the muscles of the Group 1 extremities

Muscles	Number of muscles with abnormal needle EMG findings* / total number of muscles examined in needle EMG (%)	Mean MUAP amplitude mV (median) (number)	Mean MUAP duration ms (median) (number)
TA	32/35 (91%)	7.7 ± 3.1 (8.9) (n = 22)	20.3 ± 6.7 (20) (n = 22)
MG	36/37 (97%)	7.7 ± 2.6 (8) (n = 16)	19.9 ± 6.1 (19) (n = 16)
PL	6/6 (100%)	5.7 ± 1.5 (6) (n = 3)	18.0 ± 1.7 (17) (n = 3)
VL	31/32 (97%)	9.2 ± 3.5 (8.5) (n = 16)	21.6 ± 4.7 (20) (n = 16)
IP	30/32 (94%)	7.6 ± 2.7 (8) (n = 21)	21.1 ± 3.9 (20) (n = 21)
		***p* = 0.406**	***p* = 0.191**

EMG = electromyography, MRC = Medical Research Council, MUAP = motor unit action potential, TA = tibialis anterior, MG = medial gastrocnemius, PL = peroneus longus, VL = vastus lateralis, IP = iliopsoas. *Needle EMG findings were considered abnormal if neurogenic MUAP was detected or if MUAP was not obtained during voluntary mild muscle contraction. Friedman test and Wilcoxon signed-rank test were used to compare MUAP amplitudes and duration of muscles. Boferroni correction was used for post hoc analysis. P values < 0.05 were considered statistically significant.

Table 2. Needle EMG findings in the muscles of the Group 2 extremities

Muscles	Number of muscles with neurogenic needle EMG findings / total number of muscles examined in needle EMG (%)	Mean MUAP amplitude mV (median)	Mean MUAP duration ms (median)
TA (n = 18)	12/18 (67%)	5.2 ± 3.5 (4.8)	18.5 ± 6.7 (18.5)
MG (n = 19)	9/19 (47%)	3.7 ± 3.1 (1.5)	15.7 ± 6.9 (13)
PL (n = 6)	2/6 (33%)	2.9 ± 2.9 (1.3)	14.8 ±4.2 (13.5)
VL (n = 18)	17/18 (94%)	8.9 ± 3.2 (8.3)	27.8 ±8.4 (28)
IP (n = 13)	10/13 (77%)	6.4 ± 3.2 (7)	21.5 ±6.4 (20)
		***p* = 0.002**	***p* = 0.003**
FDI (n = 32)	12/32 (38%)	2.9 ± 2.7 (1)	13.8 ± 4.3 (12.5)
APB (n = 8)	2/8 (25%)	2.3 ± 2.0 (1.3)	12.3 ± 1.9 (12)
Triceps brachii (n = 18)	8/18 (44%)	3.9 ± 3.2 (2.8)	16.3 ± 5.1 (14.5)
Biceps brachii (n = 31)	8/31 (26%)	2.6 ± 2.6 (1.5)	14.3 ±4.1 (13.5)
Deltoid (n = 20)	12/20 (60%)	4.6 ± 3.7 (4.4)	18.9 ± 5.8 (17)
		***p* = 0.005**	***p* < 0.001**

EMG = electromyography, MRC = Medical Research Council, MUAP = motor unit action potential, TA = tibialis anterior, MG = medial gastrocnemius, PL = peroneus longus, VL = vastus lateralis, IP = iliopsoas, FDI = first dorsal interosseous, APB = abductor pollicis brevis. The MUAP amplitude of the Vastus lateralis muscle was higher than the MUAP amplitude of the tibialis anterior and medial gastrocnemius and iliopsoas muscles ($p = 0.005$, $p = 0.005$, $p = 0.040$). The MUAP duration of vastus lateralis muscle was longer than the MUAP duration of tibialis anterior and medial gastrocnemius muscles ($p = 0.005$, $p < 0.001$). The MUAP amplitude of deltoid muscle was higher than the MUAP amplitude of biceps brachii muscle ($p = 0.015$) and the MUAP amplitude of triceps brachii muscle was higher than the MUAP amplitude of FDI muscle ($p = 0.040$). The MUAP duration of deltoid muscle was longer than the MUAP duration of FDI and biceps brachii muscles ($p = 0.001$, $p < 0.001$). MUAP duration of triceps brachii muscle was longer than the MUAP duration of FDI muscle ($p = 0.025$). Friedman test and Wilcoxon signed-rank test were used to compare MUAP amplitudes and duration of muscles. Boferroni correction was used for post hoc analysis. *P* values < 0.05 were considered statistically significant.

absent CMAP of the peroneal or posterior tibial nerve. Fifty-seven lower and 32 upper extremities were evaluated with needle EMG. Needle EMG could not be applied to one lower limb of a patient due to edema. Needle EMG findings of 352 muscles were analyzed. There was no upper extremity meeting the criteria of Group 1. Thirty-eight lower extremities were included in Group 1, and the needle EMG findings in the muscles of these extremities are shown in Table 1. In lower extremities included in Group 1, MUAP was not obtained in 13 (37%) of 35 tibialis anterior, 21 (57%) of 37 medial gastrocnemius, 3 (50%) of 6 peroneus longus, 16 (50%) of 32 vastus lateralis and 11 (34%) of 32 iliopsoas muscles. Table 2 shows the needle EMG findings in the muscles of the Group 2extremities. The muscles with a higher rate of needle EMG abnormalities in the upper and lower extremities included in Group 2 were deltoid (60%) and vastus lateralis (94%) muscles, respectively. The MUAP duration of the deltoid muscle was significantly longer than the MUAP duration of the first dorsal interosseous (FDI) and biceps brachii muscles ($p = 0.001$ and $p < 0.001$). The MUAP duration of the vastus lateralis muscle was longer than the MUAP duration of the tibialis anterior and medial gastrocnemius muscles ($p = 0.005$ and $p < 0.001$). Table 3 shows the needle EMG findings obtained from the analysis of two muscles together. Needle EMG was performed on the trapezius muscle in 27 of 29 patients. The needle EMG of 4 (15%) of 27 patients revealed neurogenic findings in the trapezius muscle. PSWs or FPs were seen in 22 muscles of 8 patients (Two patients had

Table 3. Needle EMG findings obtained by analyzing two muscles together in Group 2 extremities

	Number of muscles with neurogenic needle EMG findings / total number of muscles examined in needle EMG (%)
Deltoid + FDI, Deltoid + APB	7/20 (35%), 2/8 (25%)
Triceps brachii + FDI, Triceps brachii + APB	5/18 (28%), 2/8 (25%)
Biceps brachii + FDI, Biceps brachii + APB	6/30 (20%), 2/8 (25%)
VL + MG, VL + PL	8/18 (44%), 2/6 (33%)
IP + TA, IP + PL, IP + MG	8/14 (63%), 2/6 (33%), 6/13 (46%)

EMG = electromyography, MRC = Medical Research Council, TA = tibialis anterior, MG = medial gastrocnemius, PL = peroneus longus, VL = vastus lateralis, IP = iliopsoas, FDI = first dorsal interosseous, APB = abductor pollicis brevis.

post-polio syndrome). The severity score of PSWs and FPs was at most grade two. PSWs or FPs were observed in 10 medial gastrocnemius muscles of 7 patients (Two patients had post-polio syndrome). Fasciculation potentials were present in two tibialis anterior muscles of two patients. The findings of these two patients were not compatible with post-polio syndrome.

DISCUSSION

Late neuromuscular deterioration may occur later in polio survivors. Although the reason for this is unknown, excessive exercise or immune mechanisms, or chronic poliovirus infection may cause late neuromuscular deterioration [1, 5, 6]. Moreover, the death of motor neurons is thought to be faster than normal aging [7, 8]. Physical therapy strategies to protect motor neurons or axons may provide protection from neuromuscular deterioration. Excessive effort and immobility leading to weight gain can trigger neuromuscular deterioration [1, 5]. Patients should receive sufficient but not excessive physical therapy. Physical therapy strategies can be developed by considering the affected regions. Needle EMG can help identify clinically or subclinically involved regions [9]. It is not practical to examine all the muscles of a polio survivor with needle EMG as it is painful and time-consuming. Our primary goal in this study was to identify the regions involved in polio survivors by applying needle EMG to as few muscles as possible.

MUAPs are difficult to obtain from the plegic limb muscles. For this reason, we classified the extremities according to MRC scores. Among the muscles of the extremities with a mean MRC score of 2 or less, MUAP was not obtained in mostly vastus lateralis, medial gastrocnemius, and peroneus longus muscles. Therefore, iliopsoas and tibialis anterior muscles can be used primarily in detecting neurogenic MUAP. If neurogenic MUAP is present in these two muscles, it can be stated that the lumbosacral region is affected. Otherwise, other muscles can be examined with needle EMG. The low number of MUAPs obtained in some muscles of severely affected extremities was one of the limitations of this study, because the amplitude and duration of the MUAPs were analyzed from these few MUAPs.

In the Group 2 lower extremities, the muscle with the highest rate of neurogenic MUAPs was vastus lateralis followed by the iliopsoas and tibialis anterior muscles. Therefore, it may be an option to start the needle EMG study with the tibialis anterior muscle

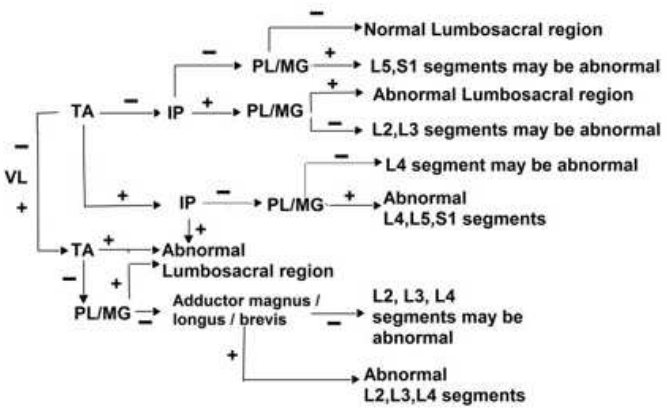

Fig. 1. Algorithm for muscle selection for needle EMG in the unaffected or mildly to moderately affected lower extremities of polio survivors. IP = iliopsoas, MG = medial gastrocnemius, PL = peroneus longus, TA = tibialis anterior, VL = vastus lateralis.

and to continue the test with the iliopsoas muscle if a neurogenic abnormality is found in the tibialis anterior muscle. However, neurogenic needle EMG abnormalities were more common in the vastus lateralis muscle. In the case of neurogenic MUAP in two different muscles of an extremity innervated by different nerves and segments, we mentioned that this region was affected in the method section. Considering this principle, the algorithm for muscle selection for needle EMG in the clinically unaffected or mildly to moderately affected lower extremity is shown in Fig. 1. A similar algorithm for the muscles of the clinically unaffected or mildly to moderately affected upper extremity is shown in Fig. 2. Among the upper limb muscles, the highest rate of neurogenic MUAPs was present in the deltoid muscle followed by the triceps brachii and FDI muscles. Since the C6 segment innervates both the deltoid and triceps muscles, it is not practical to start needle EMG with deltoid and triceps brachii muscles. We think it would be appropriate to start needle EMG with deltoid and FDI muscles. Since this study was carried out in patients without peripheral nerve damage, it is useful to consider this situation when using these algorithms. According to histopathological and clinical studies, involvement patterns by regions and muscles have been discussed in the literature [14, 15]. Similar to our study, in the literature, the involvement of the lower extremities was higher than the upper extremities [16]. It was reported that the upper lumbar spinal segments were affected more than the sacral segments [14]. Similarly, in this study, the muscles with the most neurogenic findings were the vastus lateralis, iliopsoas, and tibialis anterior muscles, respectively. In addition,

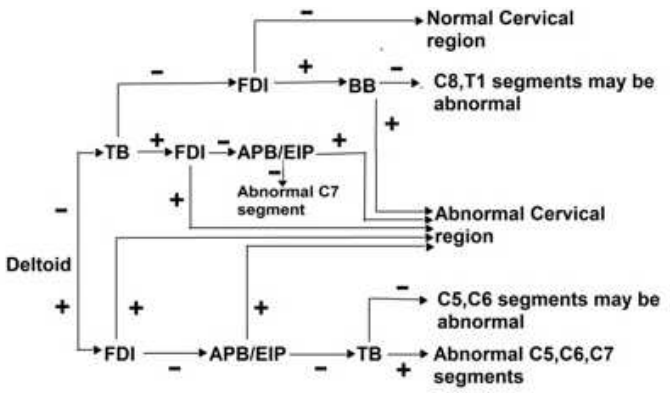

Fig. 2. Algorithm for muscle selection for needle EMG in the unaffected or mildly to moderately affected upper extremities of polio survivors. APB = abductor pollicis brevis, BB = biceps brachii, EIP = extensor indicis proprius, FDI = first dorsal interosseous, TB = triceps brachii.

the presence of abnormal posterior tibial or peroneal nerve CMAP in 59% of patients and approximately 25% of the extremities where the nerve conduction study was performed may also support that the upper lumbar spinal segments were affected more than sacral segments. While Sharrard reported the most affected muscles in the upper extremity to be deltoid, triceps brachii, and pectoralis major muscles [14], another study stated that deltoid, triceps brachii, and biceps brachii muscles were mostly affected in the upper extremity [15]. Similarly, in our study, the most affected muscle in the upper extremity was the deltoid muscle. More neurogenic abnormalities in the deltoid muscle may indicate that the upper cervical segment is more affected than the lower cervical segment [14]. Contrary to this finding, the involvement of the biceps brachii muscle was less. This may be due to the shoulder muscles being prone to damage for carrying the hanging limb [15]. Another reason may be that the length of the spinal nuclear column for each muscle is different [14, 15]. The muscle supplied by the short spinal nuclear column was more affected in poliomyelitis [14]. None of the patients had clinical involvement of cranial nerves. However, four patients had neurogenic needle EMG findings in the trapezius muscle. This finding was important since it showed that the bulbar region was affected slightly. Similar to the literature, in our study, 28% of patients and 6% of the muscles examined showed signs of active denervation [17]. Active denervation findings were mostly observed in the medial gastrocnemius muscle. We could not explain why this muscle was more involved, and this finding needs to be confirmed by further studies. This finding may be explained by the fact that the most common radiculopathies are L5 and S1 radiculopathies [5, 18].

Limitations

As we mentioned, the evaluation of a small number of MUAPs in some muscles of the severely affected extremities was one of the limitations of the study. The absence of needle EMG findings of muscles such as abductor digiti quinti, pronator teres, adductor magnus, or sternocleidomastoid was another limitation. In addition, the number of peroneus longus and abductor pollicis brevis muscles examined by needle EMG was low. We think that this algorithm can be improved with further studies in which more muscles

are analyzed with needle EMG in polio survivors. Finally, since the trapezius muscle is innervated by both the 11th cranial nerve and the cervical segments, needle EMG abnormalities in the trapezius muscle may not indicate bulbar involvement. A study of poliomyelitis involving muscles that clearly indicate bulbar involvement, such as the tongue muscle, may be interesting.

CONCLUSION

We presented an algorithm for the muscle selection for needle EMG in polio survivors. Thus, clinically or subclinically affected regions and muscles can be determined practically.

Authors' Contribution

Study Conception: HF, İÖ; Study Design: HF; Supervision: HF, ZA; Funding: HF; Materials: HF, İÖ, ZA; Data Collection and/or Processing: HF, İÖ, ZA; Statistical Analysis and/or Data Interpretation: HF, İÖ, ZA; Literature Review: HF, İÖ, ZA; Manuscript Preparation: HF, İÖ, ZA and Critical Review: HF, İÖ, ZA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Investigation of the glutathione S-transferase gene M1/T1 and angiotensin converting enzyme gene I/D polymorphism in type 1 diabetic patients and possible association with diabetic microvascular complications

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ABSTRACT

Objectives: Glutathione S-transferase (GST) polymorphism may play a role in the etiology of type 1 diabetes, as GST is involved to detoxification of reactive oxygen radicals and synthesis of proinflammatory mediators. Genetic polymorphisms in the renin-angiotensin aldosterone system, including angiotensin converting enzyme (ACE) gene insertion-deletion (I/D) polymorphism, can affect the progression of diabetes and diabetic complications. In our study we aimed to investigate the GST and ACE gene I/D polymorphism in type 1 diabetic patients for comparison with population and relationships with diabetic complications.

Methods: A total of 116 type 1 diabetic patients were included to study. ACE polymorphism analyzed in the 71 subjects and GST polymorphism analyzed in the 62 subjects as control groups. Polymorphism of DNA samples was studied by PCR technique. Results compared with control groups and studied according the diabetic complications.

Results: ACE gene DD genotype and D allele ratio in the patient group were significantly higher than control group. GST T1 and GST M1 ratios were similar between patient and control groups. ACE genotype group distributions and GST M1/T1 genotype ratios were not different in terms of obesity, glycemic control, duration of diabetes and hypoglycemia frequency and not changed according to diabetic complications.

Conclusions: DD genotype and D allele ratio in diabetic patient group were found to be significantly higher and so a significant relationship was observed between and ACE I/D gene polymorphism and type 1 diabetes. On the other hand, it was observed that ACE I/D and GST gene polymorphism did not have any significant effect on diabetic microvascular complications.

Keywords: Type 1 diabetes, ACE gene polymorphism, GST gene polymorphism, diabetic complications

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Diabetes mellitus (DM), is a major public health problem that genetic and environmental factors play a role in the pathogenesis, is increasingly prevalent throughout the world [1]. According to IDF 2017 data, there are 451 million diabetic patients worldwide and it is estimated that the number of diabetic patients will reach 693 million by 2045 [2]. Type 1 diabetes accounts for 5-10% of all diabetes cases. The rate of β-cell damage leading to the disease may vary slightly between individuals, with being faster in infants and children and slower in adults [3]. Auto-reactive T cells and autoimmunity that caused by proinflammatory cytokines and reactive oxygen radicals, are responsible for pancreatic β-cell damage. Studies in monozygotic twins have reported that environmental components may play a role in 20-60% of pathogenesis [4].

Oxidative stress resulting from impaired balance between free oxygen radicals and antioxidants plays an important role in the etiopathogenesis of type 1 diabetes and in the development of diabetic complications [5]. Glutathione, which is a major antioxidant synthesized in many cells, plays a role in the neutralization of free radicals and in the immune response. Glutathione-s-transferase (GST) represents the enzyme family that catalyzes conjugation and elimination of the substrates such as free radicals or xenobiotics. Polymorphisms in the glutathione-s-transferase mu 1 (GSTM1) and glutathione-s-transferases theta 1 (GSTT1) genes lead to decreased enzymatic activation and homozygous deletion (null) in both genes leads to a complete loss of enzyme activity. GSTM1 and T1 polymorphism are thought to be associated with many diseases such as allergy, bronchial asthma, coronary artery disease and hypertension [4, 6]. Considering the role of reactive oxygen radicals and proinflammatory mediators in the pathogenesis of pancreatic beta cell damage, GST polymorphism may play a role in the etiology of type 1 diabetes, as GST is involved in processes such as detoxification of reactive oxygen radicals and synthesis of proinflammatory mediators.

Angiotensin converting enzyme (ACE) insertion-deletion (I/D) polymorphism occurs at the intron 16 of the ACE gene, localized on chromosome 17. While, ACE activity was highest in the presence of D allele, it has lowest activity with I allele. Circulating ACE levels in plasma are 30% higher in ID heterozygotes and 60% higher in DD homozygotes when compared to II homozygotes [7, 8]. Genetic polymorphisms in the renin-angiotensin aldosterone system (RAAS), including ACE I/D polymorphism, can affect the progression of diabetes and diabetic complications [9].

In our study, it was planned to investigate the GST and ACE gene I/D polymorphism in type 1 diabetic patients and to investigate possible association with diabetic microvascular complications.

METHODS

Patients and Protocol

A total of 116 patients between 18 and 65 years of age who were followed up in the endocrinology outpatient clinic with the diagnosis of type 1 diabetes for at least one year were included in this study. Patients with malignancy or chronic disease which is not associated with diabetes and pregnant patients were not included in the study. The control group was selected for age and gender matched patients without diabetes. ACE polymorphism analyzed 71 subjects and GST polymorphism analyzed 62 subjects were included in the study as separate control groups. Patients' files, including height, weight, HbA1c, daily insulin doses, hypoglycemic episodes, presence of diabetic microvascular complications, findings at the last visits were recorded and examined in detail. Hypoglycemic events are classified as minor hypoglycemia (often: more than once per week, rare: one per week or less) and major hypoglycemia (often: more than once a year, rare: one per year or less). Patients, those with urinary microalbuminuria/creatinine levels between 30 and 100 mg were classified as microalbuminuria, those over 100 mg as macroalbuminuria, those with a glomerular filtration rate of less than 50 ml/min were classified as chronic renal insufficiency. Presence of retinopathy was assessed according to the final retinal examination. The presence of neuropathy was evaluated with complaints of the patient and physical examination findings in detail. Clinical and laboratory parameters recorded patients' blood samples was taken for genetic analysis and stored at -20 °C in EDTA.

Genetic Analysis

Genomic DNA isolation was performed using the DNA isolation kit (Dr. Zeydanli Life Sciences, Turkey) procedure from blood samples. The ACE

gene I/D polymorphism of DNA samples was detected by PCR technique. F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT 3 ', R: 5' GAT GTG GCC ATC ACA TTC GTC AGA T-3' primary was used for the ACE I/D polymorphism, F: 5 '-TGG GAC CAC AGC GCC CGC CCG CCA CTA C-3', R: 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3 ' was used as insertion region specific primer to confirm DD genotype. A 30 µL volume PCR mix was prepared for amplification of the ACE gene region from DNA samples. For each sample, the mixture contained 2.5 µl of 10X Taq polymerase buffer, 0.5 µl of dNTP mixture of 10 mM, 2 µl of MgCl2 of 25 mM, 1 µl of 10 pmol of primary pair, 0.2 µl of Taq polymerase enzyme (Bioron) and 20 µL of ddH2O. Approximately 3 µl (100 ng) DNA sample was added to the mix. The samples in the ACE DD genotype were confirmed by PCR for the second time to avoid false DD genotyping. PCR conditions consisted of denaturation at 94 °C for 1 minute followed by denaturation at 94°C for 5 minutes, 1 minute at 57 ° C for connection (63 °C to confirm the DD genotype), extension for 1 minute at 72 °C for 35 cycles and the final elongation was performed at 72 °C for 10 minutes. For PCR amplification, samples were run on 2% agarose gel electrophoresis, stained with ethidium bromide and then photographed. In the agarose gel, 190 base pairs (bp) amplification bands were observed in the samples with DD genotype, 490 and 190 bp with ID genotype and 490 bp in samples with genotype II (Fig. 1). In the second PCR analysis for DD confirmation, amplification band of 335 bp was observed in the samples with the insertion band.

In isolated DNA, the multiplex PCR method was used to determine the polymorphism of the GST M1 and GST T1 genes. Primers for forward 5'-TTCCT-TACTGGTCCTCACATCTC-3 'and reverse 5'-TCACCGGATCATGGCCAGCA-3' for GST-T1 polymorphism, forward 5'-GAACTCCCT-GAAAAGCTAAAGC-3 'and reverse 5'-GTTGGGCTCAAATATACGGTGG-3' primers for GST-M1 polymorphism and for the purpose internal controls, the primers albumin forward 5'-GCCCTCT-GCTAACAAGTCCTAC-3 'and reverse 5'-GCCC-TAAAAAGAAAATCCCCAATC-3' were used. 15, 16th PCR conditions were performed as 35 cycles of 1 min at 94 ºC (denaturation), 1 min at 58 ºC (annealing), 1 min at 72 ºC (elongation) and finally 10 min at 72 ºC (last extension) after first denaturation at 94 ºC for 5 minutes. 350 bp for albumin, 219 bp for GSTM1 and 459 bp for GSTT1 were expected to occur. The resulting products were run on ethidium bromide containing 2% agarose gel for evaluation. First, a control 350-bp product line of the albumin gene, which indicates whether the reaction has taken place, was checked. If there is no control product, PCR is repeated. The genotype determination was made as a

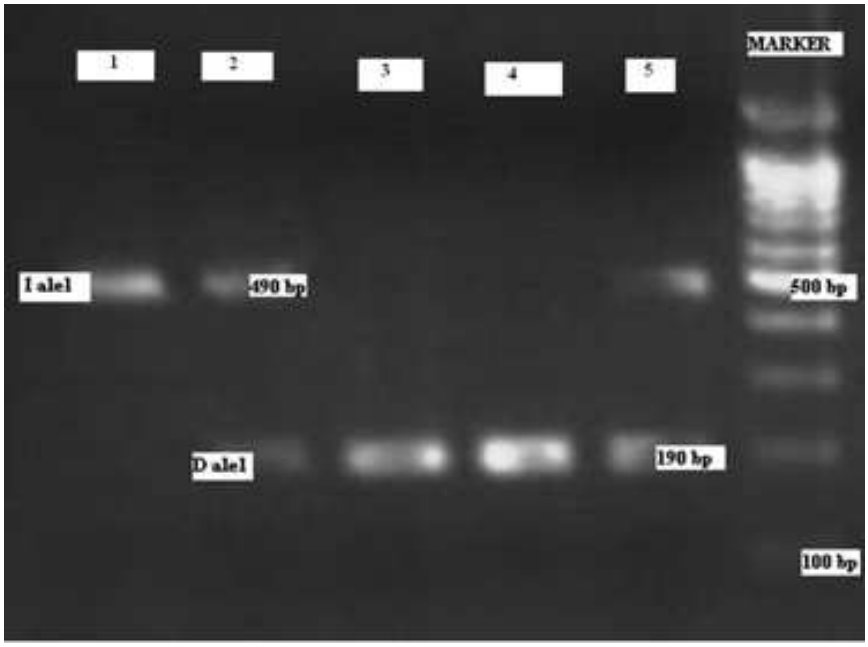

Fig. 1. Agarose gel image of PCR products made with ACE primers. The final well is a 100 bp DNA ladder (Marker), first well is case with genotype II, 2nd and 5th wells with DD genotype, 3rd and 4th wells with ID genotype.

'positive genotype' if the GSTT1 gene (459 bp) and the GSTM1 gene (219 bp) were present, or as a 'negative (null) genotype' indicating the presence of homozygote deletion.

Statistical Analysis

Demographic characteristics, HbA1c, age (years), gender, diabetes duration (years), BMI (Kg/m2) are summarized with descriptive statistics, including mean and standard deviation (values are given in parentheses) for continuous variables and frequency and percentages for categorical variables. Chi-square, Kruskal-Wallis and Mann-Whitney U variance analysis tests were used where they are convenient in the rates of D allele and I alleles in DD, ID, and II genotype groups in ACE gene polymorphism, in evaluation of GST T1 and M1 polymorphism-positive patients' rates in patients' classified according to patient / control, glycemic control and microvascular complications. Pearson and Spearman's correlation coefficients were calculated to assess the associations between variables. All analyses were performed using IBM SPSS 20™ (SPSS Inc., Chicago, IL, USA) and *p* values < 0.05 were considered statistically significant.

RESULTS

The mean age of the 116 patients was 31 ± 9.4 years and the female / male ratio was 72/44. For ACE gene polymorphism 74 control subjects and for GST polymorphism 62 control subjects were included in the study. The age and sex distribution of the patients and control groups are given in table 1. The mean duration of diabetes was 12.5 ± 8.1 years and the HbA1c level was 75 mmol/mol (9.0 ± 1.9%) in the patients. In the control group, ACE gene D/I genotype distributions were 35.1% DD, 41.9% ID and 23% II. In type 1 diabetes patients, the DD genotype was 44%, the ID genotype 45.7% and the II genotype 10.3%. The D allele rate was 66.8% in the patient group and 56.1% in the control group. The weighted genotypes and alleles were the same in both groups (ID genotype and D allele). On the other hand, DD genotype and D allele

Table 1. Clinical characteristics and genotypes of diabetic and healthy control group

		Patients n = 116	Control I n = 74	Control II n = 62	*p* value	Odds Ratio	% 95 CI
Age (years)		31.0 ± 9.4	33.0 ± 5.1	31.0 ± 5.6	0.10[a] 0.93[b]		
Gender (F/M)		72/44	37/37	38/24	0.10[a]/0.92[b]		
DD (years)		12.5 ± 8.1					
Weight (kg)		61.5 ± 9.5					
BMI (kg/m^2)		22.3 ± 3.3					
HbA1c %		9.0 ± 1.9					
mmol/mol		75					
ACE gene D/I genotype	DD %	44.0	35.1		**0.04**	1.45	0.79-2.64
	ID %	45.7	41.9			1.17	0.65-2.10
	II %	10.3	23.0			0.35	0.15-0.80
ACE gene D/I allel rates	D all %	66.8	56.1		**0.04**	1.49	0.97-2.27
	I all %	33.2	43.9		**0.04**	0.63	0.41-0.97
GST genotype	T1 %	78.4		75.8	0.73		
	M1 %	37.9		45.2	0.35		

DD = Diabetes duration, BMI = Body Mass Index, ACE = Angiotensin converting enzyme, D/I = deletion/insertion, GST = Glutathione S-transferase

Table 2. Relationships between I/D polymorphism allelic frequencies and disease releated characters in diabetic patients

		n	DD %	ID %	II %	*p* value	D allel %	I allel %	*p* value
BMI (Kg/m^2)	Normal	21	44.2	45.3	10.5	0.98	69.7	33.3	0.96
	High	95	42.9	47.6	9.5		69.8	33.2	
HbA1c *	Low	14	35.7	57.1	7.1	0.65	64.3	35.7	0.67
	High	102	45.1	44.1	10.8		67.2	32.8	
DD (years)	Short (<10)	56	44.6	39.3	16.1	0.11	64.3	35.7	0.61
	Long (>10)	60	43.3	51.7	5.0		69.2	30.8	
Minor hypoglycemia	Rare	55	40.0	50.9	9.1	0.56	65.5	34.5	0.58
	Often	61	47.5	41.0	11.5		68.0	32.0	
Major hypoglycemia	Rare	101	44.6	45.5	9.9	0.90	67.3	32.7	0.68
	Often	15	40.0	46.7	13.3		63.3	36.7	
Nephropathy	No	73	43.8	43.8	12.3	0.64	65.8	34.2	0.74
	Yes	43	44.2	48.8	7.0		68.6	31.4	
Neuropathy	No	91	47.3	42.9	9.9	0.39	68.7	31.3	0.21
	Yes	25	32.0	56.0	12.0		60.0	40.0	
Retinopathy	No	78	44.9	44.9	10.3	0.96	67.3	32.7	0.80
	Yes	38	42.1	47.4	10.5		65.8	34.2	

DD = Diabetes duration, *Low = < 7% or 53 mmol/mol, High = >7% or 53 mmol/mol

ratio in the patient group were significantly higher and II genotype and I allele ratio were significantly lower than control group (p = 0.04) (Table 1). When GST gene polymorphism was examined, T1 positivity was 78.4% and 75.8% and M1 positivity was 37.9% and 45.2% in the patient and control group respectively. There was no difference between GST T1 and GST M1 ratios between patient and control groups.

ACE gene polymorphism genotype group distributions were not different in obese and non-obese patients, in patients with and without good glycemic control, in patients with diabetes duration of less ten years or more than 10 years, and in those who had frequent hypoglycemic episodes. Similarly, when assessed for microvascular complications, in terms of genotype distribution and allele ratios, no significant difference was found according to patients with or without nephropathy, retinopathy and neuropathy (Table 2). GST gene M1/T1 genotype ratios were not different in terms of obesity, glycemic control, duration of diabetes and hypoglycemia frequency. T1 and M1 ratios were similar to those in patients with or without nephropathy, neuropathy and retinopathy (Table 3). There was no statistically significant difference between ACE genotype distribution and allele ratios and nephropathy levels. In patients with microalbuminuria, macroalbuminuria and chronic renal insufficiency, ACE genotype distributions and D/I alleles ratios were not different. Similarly, the GST T1 and GST M1 genotype ratios were similar in the nephropathy subgroups (Table 4).

DISCUSSION

Oxidative stress is defined as oxidative damage re-

Table 3. Relationships between glutathione-S-transferase genotype and disease releated characters in diabetic patients

		n	T1 %	*p* value	M1 %	*p* value
BMI (kg/m^2)	Normal	21	85.7	0.37	38.1	0.97
	High	95	76.8		37.9	
HbA1c*	Low	14	78.6	0.99	28.6	0.44
	High	102	78.4		39.2	
DD (years)	Short (< 10)	56	80.4	0.63	39.3	0.77
	Long (> 10)	60	76.7		36.7	
Minor hypoglycemia	Rare	55	76.4	0.60	36.4	0.74
	Often	61	80.3		39.3	
Major hypoglycemia	Rare	101	80.2	0.23	34.8	0.059
	Often	15	66.7		60.0	
Nephropathy	No	73	79.5	0.73	38.4	0.90
	Yes	43	76.7		37.2	
Neuropathy	No	91	80.2	0.38	38.5	0.82
	Yes	25	72.0		36.0	
Retinopathy	No	78	79.5	0.70	35.9	0.52
	Yes	38	76.3		42.1	

DD = Diabetes duration, *Low = < 7% or 53 mmol/mol, High = >7% or 53 mmol/mol

Table 4. ACE I/D polymorphism allelic frequencies and glutathione-S-transferase genotype according to the diabetic nephropathy risk

		No Nephropathy n = 73	Micro-albuminuria n = 19	Macro-albuminuria n = 11	Chronic Renal Failure n = 13	*p* value
ACE	DD %	43.8	47.4	45.5	38.5	0.97
	ID %	43.8	47.4	45.5	53.8	
	II %	12.3	5.3	9.1	7.7	
	D allel %	65.8	71.1	68.2	65.4	0.95
	I allel %	34.2	28.9	31.8	34.6	
GST	T1 poz %	79.5	68.4	90.9	76.9	0.53
	M1 poz %	38.4	31.6	45.5	38.5	0.90

ACE = Angiotensin converting enzyme, D/I = deletion/insertion, GST = Glutathione S-transferase

sulting from the disruption of the balance between reactive oxygen radicals and antioxidants. Oxidative stress plays an important role in the pathogenesis of diabetes and in the development of diabetic complications as in malignancies, cardiovascular diseases, kidney diseases and neurodegenerative diseases [1]. Glutathione is one of the most effective antioxidants to prevent cell damage caused by environmental toxins and reactive oxygen radical accumulation. Free oxygen radicals and xenobiotics are neutralized by glutathione through the glutathione-s-transferase enzyme. Glutathione-s-transferase enzyme polymor-

phisms are known to increase or decrease susceptibility to many diseases [10].

In a study comparing type 1 diabetic patients with healthy controls, the GSTT1 null genotype was found to be twice as frequent in the diabetic group, but there was no significant difference in the incidence of GSTM1 between the two groups [11]. In another study, GST T1 null genotype was found to be statistically more frequent in type 1 diabetic group compared to healthy volunteers, and the result was concluded that GST T1 null genotype increases the frequency of type 1 diabetes by 4.2-fold [10]. In the study conducted by Hori *et al*., the relationship between GST and type 2 diabetes was investigated, it is concluded that GST T1 and GST M1 null genotypes was an independent risk factor for the development of type 2 diabetes [12]. The current results were described as GST null genotypes may be associated with low antioxidant enzymatic activity [13]. On the other hand, in a study conducted in type 1 diabetic patients between 0-35 years of age and healthy volunteers in the Caucasian community, the GST M1 null genotype was associated with protection from type 1 diabetes [4]. In our study, at the aspect of GST gene polymorphism, in patient and control groups, T1 positivity were 78.4% and 75.8%, and M1 positivity was 37.9% and 45.2% respectively. GST T1 and GST M1 rates were not different between the groups. The prevalence of GST M1 null genotype frequency (45.2%) in the healthy control group was similar to that in European countries (38-62%) [14].

DD genotype was 44.0%, ID genotype was 45.7% and II genotype was 10.3% in type 1 diabetes patients included in our study. The D allele ratio was 66.8% in the patient group and 56.1% in the control group. The weighted genotypes and alleles were observed to be identical in both groups (ID genotype and D allele). On the other hand, the DD genotype and D allele ratio in the patient group were significantly higher than the control group, and the II genotype and I allele ratio were significantly lower than the control group. In the study of Hibbert *et al*., the homozygous genotype II was found to be significantly lower in long-term type 1 diabetic patients [15].

There was no significant difference between ACE gene polymorphism and glutathione-s-transferase polymorphism, obesity, duration of diabetes, glycemic control and hypoglycemia rates in type 1 diabetic patients included in our study.

Diabetes mellitus is the most common cause of chronic kidney disease. It occurs in 20-40% of diabetic patients and may progress to end-stage renal failure. Many environmental, genetic and epigenetic factors are responsible for the pathogenesis of diabetic nephropathy [3, 7]. Studies show that renal tubulointerstitial injury plays an important role in the pathogenesis and progression of diabetic nephropathy. Glutathione-s-transferases are thought to play a role in the pathogenesis of diabetic nephropathy as they are present in high concentrations in renal tubules [16]. In our study, T1 positivity was 76.7% in the diabetic nephropathy patient group while 79.5% in the non-nephropathy group and M1 positivity was 37.2% in the nephropathy patient group while 38.4% in the non-nephropathy group. The results were not statistically significant.

In the literature, the results of studies investigating the role of ACE I/D gene polymorphism in the pathogenesis of diabetic nephropathy are controversial [7]. In a meta-analysis evaluating the results of twelve studies, ACE I/D gene polymorphism was associated with end-stage renal failure in type 2 diabetic nephropathy patients, the same relationship was not observed in type 1 diabetic patients [17]. In another meta-analysis, data from 17 case-control studies in the literature were evaluated and ACE I/D polymorphism was associated with nephropathy in type 1 diabetes, particularly in Asian populations [18]. In our study, the distributions of genotype groups in diabetic nephropathy group was examined, the percentages of DD, ID and II were 44.2%, 48.8% and 7.0% respectively, and in the non-nephropathy group the percentages were 43.8%, 43.8% and 12.3% respectively. There was no statistically significant difference between the two groups in terms of ACE gene polymorphism genotype distributions.

In the study by Yang *et al*. [19], they found that GST T1 homozygote deletion is create a risk for end-stage renal failure in diabetic patients, but the same risk was not observed in GST M1 homozygote deletion. When the patients included in the study were grouped according to the presence of nephropathy, microalbuminuria, macroalbuminuria and chronic renal failure, no statistically significant differences were observed between the groups in terms of ACE gene polymorphism and glutathione-s-transferase

polymorphism.

Diabetic retinopathy is the most common cause of new blindness in the 20-74 age group in developed countries. In addition to the duration of diabetes, poor glycemic control, concomitant nephropathy, hypertension, and dyslipidemia increase the risk of developing retinopathy [3]. In the study conducted by Hovnik *et al*., the GSTM1 gene was found to be associated with diabetic retinopathy. As a result of this study, the authors suggested that GSTM1 gene deletion is protective for diabetic retinopathy [20]. Bekris *et al*. [4], found that GSTM1 gene deletion is protective against the development of type 1 diabetes in children aged 14-20 years. It is thought that the protective effect of GSTM1 null genotype on diabetic retinopathy in type 1 diabetic patients could be explained by the upregulation of the other antioxidant enzymes such as manganese superoxide dismutase with GSTM1 gene deletion [21]. In our study, while T1 positivity was 76.3% in the diabetic retinopathy group, it was 79.5% in the non-retinopathy group and, while M1 positivity was 42.1% in the retinopathy group, it was 35.9% in the non-retinopathy group. There was no statistically significant difference between the groups.

No significant association was found between ACE I/D polymorphism and the presence of diabetic retinopathy in patients with type 1 diabetes in the Caucasian race [22]. In our study, DD, ID and II genotype groups' percentages of ACE gene polymorphism were 42.1%, 47.4% and 10.5% in diabetic retinopathy group, respectively, whereas it was 44.9%, 44.9% and 10.3% in non-retinopathy group respectively. No statistically significant difference between the two groups in terms of ACE gene polymorphism genotype distributions was observed.

Diabetic peripheral neuropathy is asymptomatic up to approximately 50% in diabetic patients [3]. In type 1 diabetic patients, good glycemic control can effectively prevent diabetic peripheral neuropathy and cardiac autonomic neuropathy [23]. Babizhayev *et al*. [24] suggested that polymorphism in genes encoding antioxidant enzymes might cause genetic susceptibility in type 1 diabetic patients. In our study, while T1 positivity was 72.0% in the diabetic neuropathy patient group, it was 80.2% in the non-neuropathy group and while M1 positivity was 36.0% in the neuropathic patient group and 38.5% in the neuropathy group, respectively. There was no significant difference between the groups. Peripheral nerve damage in diabetic patients has been associated with polyol accumulation, AGE products and oxidative stress. One of the mechanisms involved in pathogenesis is that hyperglycemia stimulates NADP oxidase by raising tissue angiotensin levels by RAAS activation, thereby increasing oxidative stress and vascular injury [25]. In our study, the percentages of DD, ID and II genotype groups of ACE gene polymorphism were 32.0%, 56.0% and 12.0% in the diabetic neuropathy group, respectively, and 47.3%, 42.9% and 9.9% in the non-neuropathy group, respectively. No statistically significant difference in terms of polymorphism genotype distributions was observed.

In diabetic patients with DD genotype, serum ACE levels are higher and increased RAS activity in diabetic patients is associated with increased ACE levels. The high rate of DD genotype in the patient group in our study supports this. It is possible that increased RAS activity may increase the progression of nephropathy, and some of the cited studies have suggested a relationship between the occurrence of complications, particularly nephropathy, and the DD genotype. In our study, however, DD genotype was not found to be associated with complications. This situation shows that other factors besides the increased RAS activity also affect the complications.

CONCLUSION

In this study, the relationship between GST genotype and ACE I/D gene mutation and age, gender, duration of diabetes, presence of hypoglycemia and diabetic microvascular complications such as nephropathy, retinopathy and neuropathy in type 1 diabetic patients was investigated. As a result of our study, DD genotype and D allele ratio in diabetic patient group were found to be significantly higher than that of healthy control group, and II genotype and I allele ratio were found to be significantly lower in diabetic patients, and a significant relationship was observed between and ACE I/D gene polymorphism and type 1 diabetes. On the other hand, the same relationship was not detected in the GST gene polymorphism. However, as a result of our study, it was observed that ACE I/D and GST gene polymorphism did not have any significant effect on diabetic mi-

crovascular complications. Considering the different results on the subject, studies in the literature suggest that studies involving a higher number of patient groups will be important for the detection of susceptibility genes and for assessing the risk of complications in type 1 diabetic patients in which complex factors play a role in etiopathogenesis.

Ethics Approval and Consent to Participate

Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the institutional ethics committee.

Authors' Contribution

Study Conception: SC,; Study Design: SC,; Supervision: CE,; Funding: SC; Materials: SC; Data Collection and/or Processing: SC, ÖÖG, PŞ, AD; Statistical Analysis and/or Data Interpretation: SC, MK; Literature Review: SC, PŞ; Manuscript Preparation: SC, NK, PŞ and Critical Review: NK, CE.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The in vitro cytotoxic, genotoxic, oxidative damage potential of enoxaparin sodium in human peripheral blood mononuclear cells

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ABSTRACT

Objectives: Enoxaparin sodium, low-molecular weight heparin (LMWH) indicated for the prophylaxis deep vein thrombosis. As far as we know, its cytotoxic, genotoxic and oxidative effects have never been studied on any cell lines. The purpose of the present study is to evaluate the in vitro cytotoxic, genotoxic damage potential and antioxidant/oxidant activity of enoxaparin sodium on primary human whole blood cultures.

Methods: After exposure to different doses (from 0.5 to 100 mg/L) of enoxaparin sodium, cell viability was assessed by the cytotoxicity tests including MTT (3, (4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide) and lactate dehydrogenase (LDH) release assays. The antioxidant activity was measured by the total antioxidant capacity (TAC) and total oxidative stress (TOS) parameters. To determine the genotoxic damage potential, the rate of chromosomal aberrations (CAs) and 8-oxo-2'-deoxyguanosine (8-oxo-dG) levels were also assessed.

Results: Cytotoxicity assays showed that treatment with enoxaparin sodium caused significant decreases in the cellular viability in a clear dose-dependent manner. Also, it was found that enoxaparin sodium did not alter the TAC and TOS levels. The genotoxicity assay showed that the formation of CAs was not observed in the lymphocytes. Likewise, the levels of 8-oxo-dG did not change in treated cultures as compared to control values.

Conclusions: Enoxaparin sodium appeared to exhibit cytotoxic but not oxidative and genotoxic damage potentials in cultured human blood cells.

Keywords: Enoxaparin sodium, low-molecular-weight heparin, cytotoxicity, oxidative stress, DNA damage, human peripheral blood mononuclear cells

Venous thromboembolism (VTE), one of the most causes of cardiovascular diseases, is the leading preventable cause of mortality and morbidity in inpatients, the incidence of which is estimated to double in the future [1]. Heparins are commonly used drugs for the prophylaxis and treatment of VTE [2]. Low-Molecular-Weight Heparins (LMWHs) exert more predictable effects and require less coagulation level monitoring than that of unfractionated heparin (UFH) [2]. Enoxaparin sodium, a type of LMWHs, is often preferred due to its high bioavailability in the subcutaneous form [3, 4].

Anticoagulants have wide usage for preventing and treating VTE. Surveys have shown that the most widely used agent in therapeutic anticoagulation is LMWHs [5-9]. LMWHs are an important class of antithrombotic medications and derived from UFH by depolymerization procedure [10]. LMWHs have wide

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usage in comparison with UFH because of its advantages as pharmacokinetic profile and ease of use [11, 12]. The mean molecular weight of LMWHs is 4.0-6.0 kD. It varies by manufacturing process, thus it was influenced by its in vivo properties including pharmacokinetics, bioavailability, and plasma half-life [13, 14].

Among the LMWHs, enoxaparin sodium is one of the most widely prescribed agents and has been used since 1993 [5, 7, 15]. Enoxaparin sodium indicated for the prophylaxis deep vein thrombosis. It is used commonly in orthopedic clinical practice. Interestingly, the development of biosimilar versions of enoxaparin increased medical concerns about their efficacy and safety [16]. At this point, there is not sufficient data for elucidating the toxicity potentials of these bio-similar versions of the anticoagulants.

The enoxaparin sodium which is the most used agent in the orthopedic clinic approach. This agent is used by orthopedic patients during the long-time and sometimes a few times a day. Therefore, this study was aimed to research the potentials of the enoxaparin sodium on the human peripheral blood mononuclear cells (PBMCs) via determining cytotoxic, genotoxic and oxidative damage potentials for the first time.

METHODS

Chemicals and Reagents

Enoxaparin Sodium (Oksapar 6000, IU/0.6 mL; Koçak Farma, Turkey) was supplied in prefilled glass syringes from the manufacturer and diluted with the cell culture medium. All measurements by devices were performed according to the protocol of the manufacturer.

Mononuclear Cell Isolation

Whole blood samples were obtained from 5 donors (healthy, 22-25 aged, non-smoking, with no genotoxic agent history, n=5). The equal volume of Phosphate-buffered Saline (PBS) was used to dilute the blood sample. Then, the blood samples were layered on Ficoll-Hypaque-Plus (GE Healthcare Biosciences Corp., Piscataway, NJ, USA) and centrifuged at 400×g for 30 minutes at 18-24°C.

The PBMC layer was carefully removed, transferred, and washed with three volumes of PBS for twice and re-suspended in RPMI-1640 media (GIBCO, USA) including 10%, v/v Fetal Bovine Serum (FBS) (Sigma, USA) and 1% penicillin/streptomycin (Sigma, USA). PBMCs were counted using haemo-cytometer and 105 cells incubated each wheel during the experiment. In all cell viability, oxidative and genotoxic damage assays, the cultures without enoxaparin sodium were chosen as negative control groups [control (-)]. The cell cultures treated with Triton X-100 (1%, Sigma-Aldrich) were chosen as a positive control group [control (+)] for MTT and LDH release assays. In addition, ascorbic acid (10 mg/L, Sigma-Aldrich) and hydrogen peroxide (25 mg/L, Sigma-Aldrich) treated cells were chosen as the positive control groups in the analyses as Total Oxidant Status (TOS) and Total Antioxidant Capacity (TAC). Mitomycin C (10 mg/L, Sigma-Aldrich, USA) treated cells was also chosen as a positive control group in chromosomal aberrations (CAs) and 8-OH-dG assays [17].

MTT Assay

MTT assay is one of the most used colorimetric assays for detecting cell viability. The cells were placed in 96-well plates to perform the assay. They were incubated in carbon dioxide incubator (37 °C, 5% CO2) and treated with different concentrations of enoxaparin sodium (as 0.5, 1, 2.5, 5, 10, 25, 50 and 100 mg/L) for 72 h. MTT Cell Growth Assay Kit (Merck Millipore, USA) was used to perform MTT assay. 10 µl of MTT solution was added to well to incubate for 4h. 100 µl of acid-isopropanol (isopropanol with 0.04 N HCl) was added for the dissolving procedure of the formazan crystals. Then, a microplate reader (Synergy-HT; BioTek Winooski, VT, USA) was used to detect the optical density at 570 nm. The cell viability was expressed in percentages of viable cells.

LDH Release Assay

LDH assay is based on the measurement of the amount of released LDH from cells is to assess cell death [18]. The CytoSelect® LDH cytotoxicity assay kit was used to perform the assay. The cells were placed in 96-well plates and they were incubated in carbon dioxide incubator (37 °C, 5% CO2). They treated with enoxaparin sodium at selected concentrations for 72 h. 96-well plate was centrifuged for 5 minutes at 400×g. 90 µl of the supernatant with negative

control, positive control, and test groups was transferred to new plates. 10 µl of LDH as cytotoxicity assay reagent was added into well. They were incubated for 30 minutes at 37 °C. A microplate reader (Synergy-HT; BioTek Winooski, VT, USA) was used to measure the absorbance at 450 nm.

Total Antioxidant Capacity (TAC) Assay

TAC assay enables measurement of the capacity of all types of antioxidants in experimental samples. In brief, in this assay, the existing antioxidants in the test sample reduce 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid cation radical (ABTS) which is dark blue/green colored to the reduced form of colorless ABTS. Due to this reduction, the alteration of the absorbance values at 660 nm has a relationship with the total antioxidant amount of the sample [19, 20]. The plasma samples, obtained from the whole blood cultures after 72h incubation with enoxaparin sodium at different concentrations, were examined using the TAC assay kit (Rel Assay Diagnostics®, Turkey). 30 µl of sample and 500 µl of reagent 1 were mixed. After 30 seconds, the absorbance was measured at 660 nm by using a spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). 75 µl reagent 2 was added and after 10 minutes incubation at room temperature condition, the absorbance was measured at 660 nm by using a spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). The same procedure was carried out using deionized water and standard as well as examples. Trolox equivalent (1 mmol / L), a vitamin E analog, was used as standard. Results were expressed in mmol / L (Erel 2004).

Total Oxidant Status (TOS) Assays

TOS assay provides the measurement of oxidants existing in test samples which oxidize the complex containing ferrous ion-chelator to ferric ion. The ferric ion procures colorful complex by chromogen in the medium at acidic conditions. The color intensity determined as spectrophotometrically has a relationship with the total amount of oxidants [21]. The plasma samples, obtained from the whole blood cell cultures after 72h incubation with enoxaparin sodium at different concentrations, were examined using the TOS assay kit (Rel Assay Diagnostics®, Gaziantep, Turkey). 75 µl of sample and 500 µl of reagent 1 were mixed. After 30 seconds, absorbance at 530 nm was measured using a spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). 25 µl of reagent 2 was added and incubated for 10 minutes at room temperature condition. A second absorbance measurement was performed at 530 nm by using spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). Hydrogen peroxide (20 mmol / L) was used as a standard. Results were expressed in µmol H2O2 Equiv/L [21, 22].

Chromosomal Aberrations (CA) Assay

Whole human blood samples were treated with enoxaparin sodium at selected concentrations. They were cultured for 72h. Before two hours of harvesting, 0.02 µg/ml of Colchicine (Sigma, USA) was given into the culture. Hypotonic treatment (0.075 M KCl/37.4°C) was performed. The fixation (methanol plus acetic acid) three times, cells were harvested by centrifugation. The slides were prepared from each fixed-cell suspension and they were air-dried. Then, in phosphate buffer (pH 6.8), all slides were stained with Giemsa. To score for each treatment, 30 well-spread metaphases were analyzed for chromosome aberration. All the aberrations (as chromatid/chromosome gap or/and chromatid/chromosome break) were determined and classified according to the criteria of Environmental Health Criteria 46 for Environmental Monitoring of Human Populations [23, 24].

Determination of 8-OH-dG Level

DNA oxidation was determined by detecting the amount of 8-OH-dG adducts. DNA was digested in the incubation period with DNase I, alkaline phosphatase, and endonuclease. The high-performance liquid chromatography (HPLC) was used to measure the amount of 8-OH-dG with electrochemical detection as described in the literature [25]. Waters S-3 4.6×150 mm column [with 5% methanol/95% 100 mM sodium acetate buffer (pH 5.2)] at a flow rate of 1.0 mL/min were used to separate the compound. The four electrochemical detector channels (at -100, 250, 475, and 875 mV) were set up.

Statistical Analysis

SPSS software (version 20.0, SPSS, Chicago, IL, USA) was used to perform statistical analysis. The Kruskall Wallis test was used for the statistical analysis of values. Statistical decisions were given with significance levels of 0.05 and 0.005.

RESULTS

LDH and MTT release assays were used to determine the cell viability of human PBMCs against enoxaparin sodium. The results for cytotoxicity measured by MTT assay were shown in Fig. 1. The cultured human PBMCs exposed to relatively low concentrations of enoxaparin sodium (as 0.5, 1, 2.5, and 5 mg/L) did not exhibit any important changes for cell viability over 72h ($p > 0.05$). However, 10 and 25 mg/L concentrations of enoxaparin sodium caused a weak cytotoxic effect on human PBMCs and there is no statistical significance ($p > 0.05$). Enoxaparin sodium leads to a decrease in the proliferation of human PBMCs, at higher concentrations than 25 mg/L (50 and 100 mg/mL) when compared to the control group ($p < 0.05$). The cytotoxicity of enoxaparin sodium on cultured human PBMCs by measuring the amount of intracellular LDH release was assessed in Fig. 2. LDH levels were not affected by low doses of enoxaparin sodium, only 50 and 100 mg/L of enoxaparin sodium caused to the significant increase of LDH level ($p < 0.05$). IC20 and IC50 values of enoxaparin sodium in PBMCs were determined according to the results of

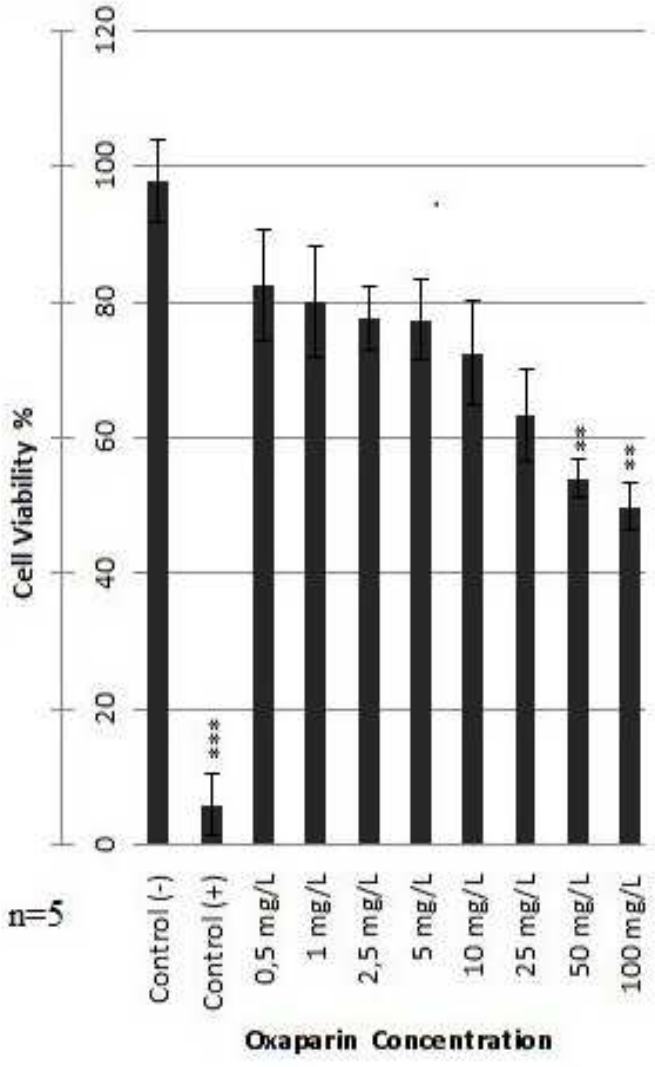

Fig. 1. Cell viability rates of human PBMCs after exposure to various enoxaparin sodium concentrations for 72h. The cells grown in media without enoxaparin sodium was used as control (-) group. The cell cultures treated with Triton-X (1%) was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **$p < 0.05$ and ***$p < 0.001$ (n = 5).

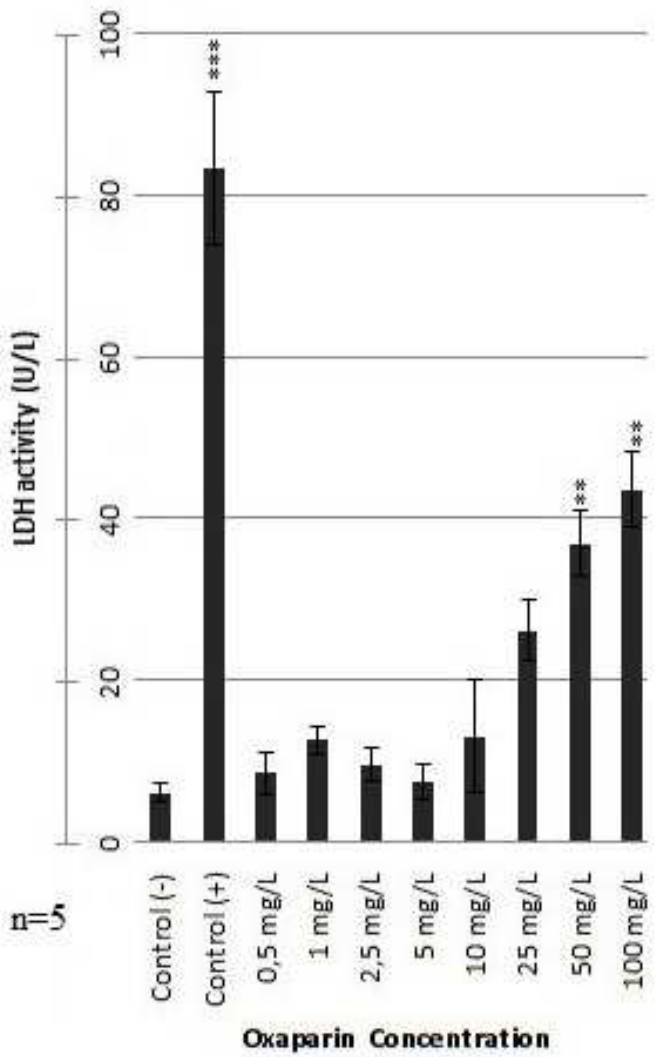

Fig. 2. LDH levels of human PBMCs treated with different concentrations of enoxaparin sodium for 72 h. The cells grown in media without enoxaparin sodium was used as control (-) group. The cell cultures treated with Triton-X (1%) was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **$p < 0.05$ and ***$p < 0.001$ (n = 5)

MTT assay and calculated as 25,647 and 93,416 mg/L, respectively.

The oxidative status effects of various enoxaparin sodium concentrations in human PBMCs cultures were evaluated by using TAC and TOS analysis. 0.5, 1, 2.5, and 5 mg/L concentrations of enoxaparin sodium exposure did not change TAC levels; but 10, 25, 50, and 100 mg/L concentrations of enoxaparin sodium treatment caused to a slight decrease of TAC levels, as shown in Fig. 3. Enoxaparin sodium did not cause to increase of TOS levels in cultured human PBMCs at all tested concentrations. Also, it did not change significantly both TAC and TOS levels when compared to the negative control groups (p >0.05). The genotoxic effects of enoxaparin sodium were analyzed by CA assay in human PBMCs and it is shown in Fig. 5. All tested concentrations of enoxaparin sodium did not cause significant increases in the number of observed CAs (p > 0.05). Similarly, enoxaparin sodium concentrations did not show an increase in the levels of 8-OH-dG when compared to the control group as seen in Table 1.

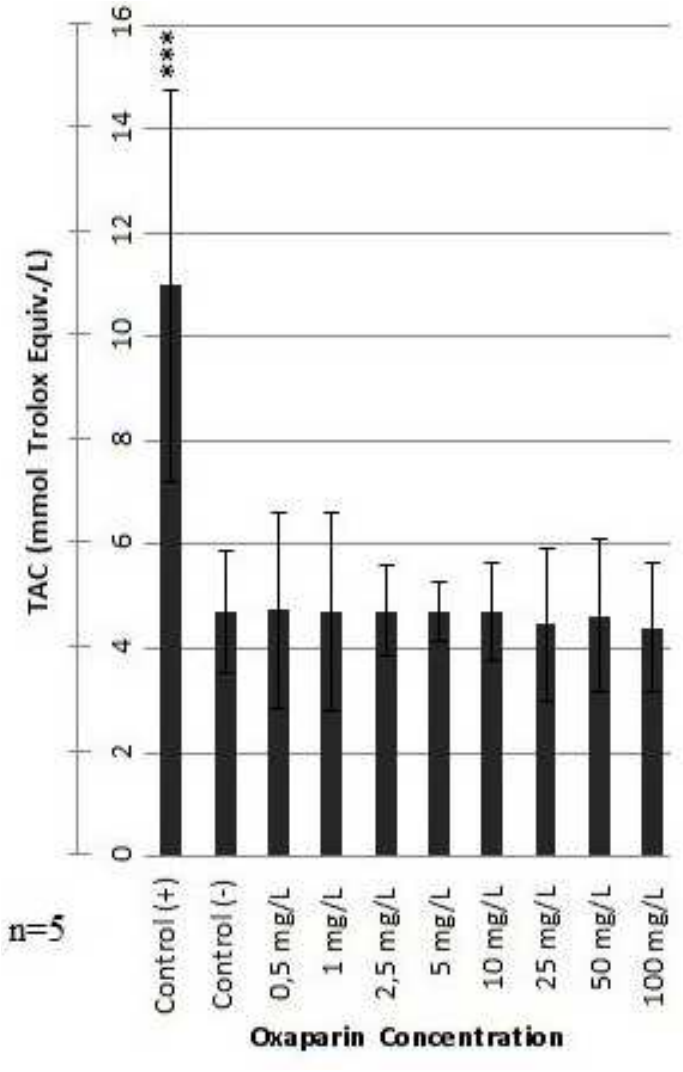

Fig. 3. The levels of TAC in cultured human PBMCs exposed to enoxaparin sodium for 72 h. The cells grown in media without enoxaparin sodium was used as control (-) group. Ascorbic acid (10 mg/L) treated cell culture was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **p < 0.05 and ***p < 0.005 (n = 5).

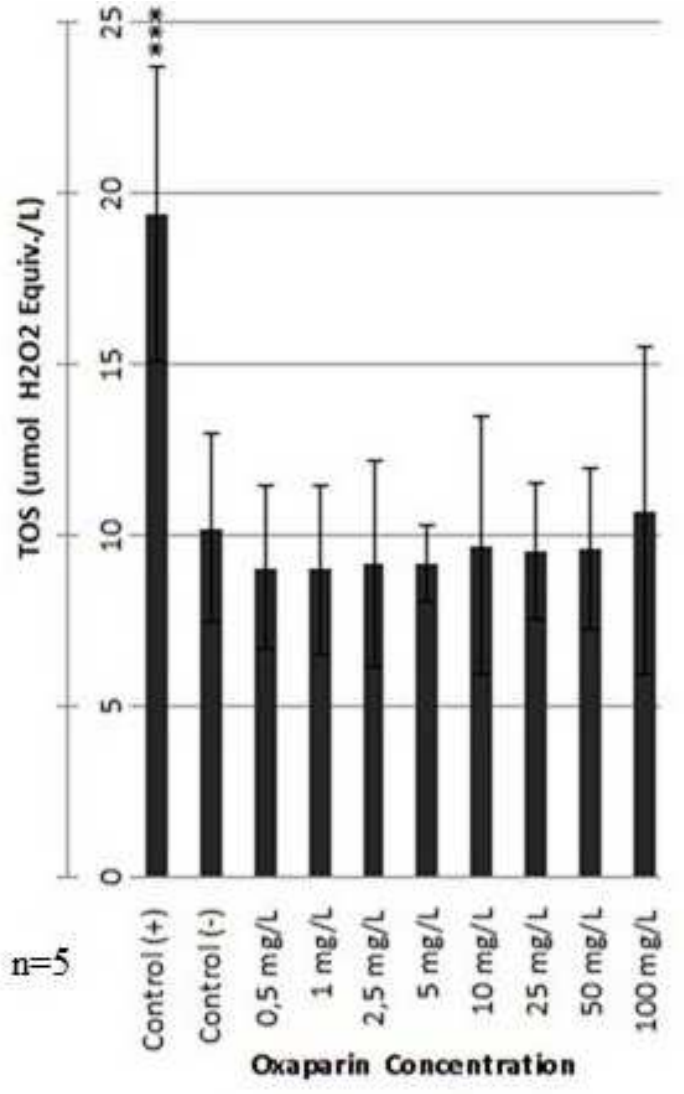

Fig. 4. The levels of TOS in cultured human PBMCs exposed to enoxaparin sodium for 72 h. The cells grown in media without enoxaparin sodium was used as control (-) group. PBMCs treated with hydrogen peroxide (25 mg/L) was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **p < 0.05 and ***p < 0.005 (n = 5).

Fig. 5. Representative images of chromosomal aberrations observed in cultured human PBMCs exposed to different concentrations of enoxaparin sodium for 72h. a) Control (-): undamaged chromosomes are seen, b) treatment with IC50 concentration of enoxaparin sodium, c) treatment with MMC, chromosomal aberrations indicated by arrows (n = 5).

Table 1. The 8-OH-dG levels in cultured PBMCs after exposure to enoxaparine sodium concentrations for 72h

Groups	CAs/cell	8-oxo-dG level (pmol/micro g DNA)
Control (-)	0.20 ± 0.03	0.81 ± 0.08
Control (+)	2.59 ± 0.18*	4.22 ± 0.35*
0.5 mg/L	0.20 ± 0.02	0.73 ± 0.06
1 mg/L	0.22 ± 0.03	0.76 ± 0.09
2.5 mg/L	0.16 ± 0.02	0.75 ± 0.08
5 mg/L	0.18 ± 0.03	0.72 ± 0.10
10 mg/L	0.20 ± 0.04	0.79 ± 0.07
25 mg/L	0.24 ± 0.03	0.81 ± 0.12
50 mg/L	0.26 ± 0.04	0.83 ± 0.07
100 mg/L	0.28 ± 0.03	0.88 ± 0.10

*Symbol presents significant statistical difference from the control (-) group. The cells grown in media without enoxaparin sodium was used as control (-) group. Mitomycin C (10 mg/L) treated cell group was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Enoxaparin sodium concentrations did not show an increase in the levels of 8-oxo-dG on cultured human lymphocytes when compared with the control group

DISCUSSION

In this study, enoxaparin sodium was subjected to an in vitro toxicity evaluation in order to reveal its safety profile on human PBMCs. The performed MTT assay in this investigation showed that higher concentrations of enoxaparin sodium lead to a decrease in the viability of PBMCs. LDH release assay gave similar results with MTT assay. In fact, LDH activity reached the highest level at the concentrations as 25, 50, and 100 mg/L of enoxaparin sodium. In accordance with our findings, it was reported that enoxaparin sodium (lower concentrations than 0,024 mg/ml or 2.4 IU/mL) caused inhibition of proliferation whereas higher concentrations impaired cell growth in the dose-dependent manner [26]. Also, it was found that heparin and its low molecular-weight fragments could inhibit the proliferation of vascular smooth muscle cells (SMCs) both in vivo and in vitro conditions [27-31]. On the contrary, it was previously executed that enoxaparin did not show any significant effect on the proliferation of PBMC [32, 33].

In this study, TOS and TAC assays were used to detect the oxidative status of enoxaparin sodium. Results obtained from the assays demonstrated that enoxaparin sodium caused a slight decrease in the

TAC level but no significant changes in the TOS level. There is no study about the oxidative potential of enoxaparin sodium. It has been reported that LMWH reduced oxidative stress in hemodialysis patients [34]. Additionally, it was propounded the presence of protective effects by LMWH on oxidative injuries. Again, LMWHs led to decreases in lipid peroxidation in cardiac and hepatic tissues [35].

Our study also evaluated the genotoxicity of enoxaparin sodium on human PBMCs using CA and 8-OH-dG level assays. These assays revealed that enoxaparin sodium did not lead to increasing of 8-OH-dG levels and CA frequency. The results indicated that enoxaparin sodium might not be genotoxic on human PBMCs. On the contrary, the genotoxic damage potentials of heparin, dalteparin, enoxaparin, and nadroparin were reported using micronucleus (MN) assay. Moreover, all these tested agents caused a significant increase in the rates of MN in a dose-dependent manner for rat embryonic blood cells as well as inducing morphologic abnormalities [36].

CONCLUSION

The results of this study demonstrate that enoxaparin sodium does not cause genotoxicity but high concentrations (25-100 mg/L) of enoxaparin sodium exhibit cytotoxic action on PBMCs. Thus, it is suggested that the dose management should be reconsidered due to possible cytotoxic effects of enoxaparin sodium. Also, further in vivo investigations are required in order to evaluate the safety of enoxaparin sodium.

Authors' Contribution

Study Conception: KY; Study Design: KY; Supervision: KY; Funding: ŞEÖ, ŞE; Materials: KY; Data Collection and/or Processing: KY; Statistical Analysis and/or Data Interpretation: KY; Literature Review: KY; Manuscript Preparation: KY and Critical Review: KY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Medial insufficiency in postoperative weight-bearing radiographs in supination-external rotation type 4 bimalleolar ankle fractures: is the Lauge-Hansen classification insufficient in predicting medial soft tissue damage?

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ABSTRACT

Objectives: According to the Lauge-Hansen classification, supination external-rotation (SER) type 4 fractures should be accompanied by medial malleolar fracture or deltoid ligament injury. The aim of the study was to investigate medial insufficiency rates in postoperative weight-bearing radiographs in SER type 4 bimalleolar fractures.

Methods: The files of the patients who were operated with the diagnosis of SER type 4 bimalleolar ankle fracture between 2017-2020 were evaluated retrospectively. Thirty-seven cases (15 males and 22 females) were included in the study. The data based on the evaluation of the weight-bearing radiographs of the patients taken in the postoperative 1st year were examined statistically.

Results: The injury mechanism was sports injury in 17 (45.9%) cases, traffic accidents in 8 (21.6%) cases, falling in 9 (24.3%) cases, and falling from height in 3 (8.2%) cases. The preoperative tibiofibular distance was 6.05 ± 1.86 mm, and the postoperative tibiofibular distance was 4.19 ± 0.40 mm (p = 0.001). The preoperative tibiofibular overlap was 5.03 ± 2.93 mm, and the postoperative tibiofibular overlap was 8.62 ± 1.04 mm (p = 0.001). The postoperative medial clear space was 4.11 ± 0.57 mm. Postoperative medial clear space of 5 mm and higher was determined in 7 (18.9%) cases.

Conclusions: In SER type 4 bimalleolar fractures with a large medial malleolar fragment fracture, weight-bearing radiographs may show an increase in medial clear space. This means that a medial malleolar fracture in bimalleolar fractures may be also accompanied by deltoid ligament injury. The Lauge-Hansen classification system may be insufficient to identify a medial ligament injury.

Keywords: bimalleol, fracture, supination-external rotation, Lauge-Hansen, deltoid ligament

The Lauge-Hansen classification system is often used for ankle fractures. Supination-external rotation (SER) injury is the most common type among all ankle fractures (accounting for up to 85%) [1]. In SER type 4 fractures, a lateral malleolar fracture is accompanied by either a medial malleolar fracture or a deltoid ligament rupture, which are treated surgically [2, 3].

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The purpose of ankle fracture treatment is to correct tibiotalar joint congruity and stability; thus, identifying ligament injuries in the formation of an ankle fracture is critical for treatment decisions [4]. In the literature, there are findings suggesting that in SER type 4 bimalleolar fractures, a medial malleolar fracture may be accompanied by a deltoid ligament injury [5-8]. However, these studies reported this either as a MRI finding or associated it with small fragment fractures at the collicular level at which the deltoid ligament is attached [5-8].

The Lauge-Hansen classification may not provide sufficient information to predict the fracture mechanism and soft tissue injuries [5, 9]. We suggest that SER type 4 bimalleolar fractures according to the Lauge-Hansen classification as well as large fragment medial malleolar fractures in the supracollicular region may be concomitantly accompanied by deltoid ligament insufficiency depending on the mechanism and transferred energy. Thus, we aimed to investigate the presence of cases with increased medial clear space in weight-bearing mortise radiographs taken at postoperative 1st year among the cases with SER type 4 injury who were operated with the diagnosis of a bimalleolar fracture.

METHODS

The files of the patients who were operated with the diagnosis of ankle fracture between 2017-2020 were evaluated retrospectively. The preoperative computed tomography (CT) images were examined, and only the images of the cases with bimalleolar fracture were evaluated. Based on the CT images, the cases (lateral+medial) were categorized by two surgeons according to the Lauge-Hansen classification. In the case of disagreement between two observers, a decision was made after examination of the images by two more persons [10]. Pediatric cases, cases with pilon fractures, cases with an additional injury, cases with a neuropathic disease, cases with neurovascular injury, cases with a pathologic fracture, cases with other type of rotational ankle fracture, cases with concomitant posterior malleolar fracture (including avulsion fracture), cases who undergone two-stage surgical protocol with external fixator, cases with postoperative tibiofibular joint incongruence, cases with insufficient postoperative reduction, and cases with a small medial malleolar fragment fracture (at collicular level) were not included in the study. The files of 37 patients diagnosed with supination-external rotational type 4 bimalleolar fracture (lateral+medial) based on the Lauge-Hansen classification were included in the study.

All the cases were operated in supine position under spinal anesthesia. In all the cases, the fracture was fixed with both lateral and medial skin incision. For stabilization of the lateral malleolus, posterolateral anatomic distal fibula plate (by TST - Turkish Spinal Trauma company) was used. The medial malleolar fracture was fixed using 2 malleolar screws or tension band technique. In the cases having syndesmotic insufficiency determined in external rotation test after the fracture stabilization, the syndesmotic screw was passed through the appropriate hole on the plate (at least 3 cortex) to provide syndesmotic stability. Then, the operation was ended by checking the stability of the syndesmosis with external rotation testing. A hemovac drain was placed in all the cases, which was removed within 48 hours after the operation. All the cases were immobilized in a short leg splint for 7-14 days, which then was removed to allow the joint movements. They were allowed for non-weight bearing walking for up to 6 weeks after the operation; then they were allowed for weight-bearing as tolerated. The weight-bearing was increased over time, and full weight-bearing was allowed according to radiography or clinical union findings.

The postoperative direct radiography and fluoroscopy images were evaluated based on the criteria of anteroposterior tibiofibular overlap (TFO) (normal > 6 mm), tibiofibular clear space (TCS) (normal < 6 mm) and medial clear space (MCS) (normal < 5 mm). The cases with values outside normal values for these criteria were considered as having tibiofibular incongruence [11]. In the evaluation of the intraoperative syndesmotic joint, the same criteria were used. The postoperative radiographic measurements were based on weight-bearing radiographs.

The monitoring software, INFINITT PACS (Picture Archiving and Communication Systems) version 3.0.11.4(BN13), which was available in our hospital, was used for the pre-operative CT and post-operative radiographic measurements. The postoperative measurements were evaluated based on the radiographs

taken in the 1st year after operation. The measurements were performed 2 surgeons who did not perform the operations, and then they were averaged. The numerical data obtained based on these measurements were used as data in statistical calculations.

The cases whose the fracture line was located on both colliculus based on the preoperative computed tomography images were included in the study. The cases whose fracture line was at or below colliculus level (e.g.: small fragment oblique fractures) were not included in the study (Fig. 1).

The cases were operated by 3 different surgeons. All the surgeons had more than 4 years of experience in trauma surgery. The total number of lateral malleolar fracture operations performed by them in the last 3 years was more than 15 per year.

The information of the patients such as age, sex, fractured side, injury mechanism, fracture type according to the Lauge-hansen classification, lateral malleolar fracture type according to the Denis-weber classification, the presence of preoperative syndesmosis dysfunction, the use of a syndesmotic screw, fracture union times, preoperative and postoperative TFO values, preoperative and postoperative TCS values and postoperative MCS values were recorded and used in the statistical analysis.

Statistical Analysis

The conformity of the data with normal distribution was tested with the Shaphiro wilk test, the two independent group comparisons of parameters with normal distribution was examined with the Student t test, and the two independent group comparisons of parameters without normal distribution was examined with the Mann Whitney U test. The relationship structure of categorical data was analyzed with the Fisher Exact and Pearson Chi-Square tests. As a descriptive statistic, mean ± standard deviation values were given for the numeric variables, while number and % values were given for the categorical variables. The statistical analyses were performed using SPSS Windows version 23.0 package program, and a result with $p < 0.05$ was considered to be statistically significant.

RESULTS

Fifteen (40.5%) males and 22 (59.5%) females patients were included in the study. The average age was 49.30 ± 13.50 years. The fracture affected the right extremity in 20 (54.1%) cases and the left extremity in 17 (45.9%) cases. The injury mechanism was sports injury in 17 (45.9%) cases, traffic accidents in 8

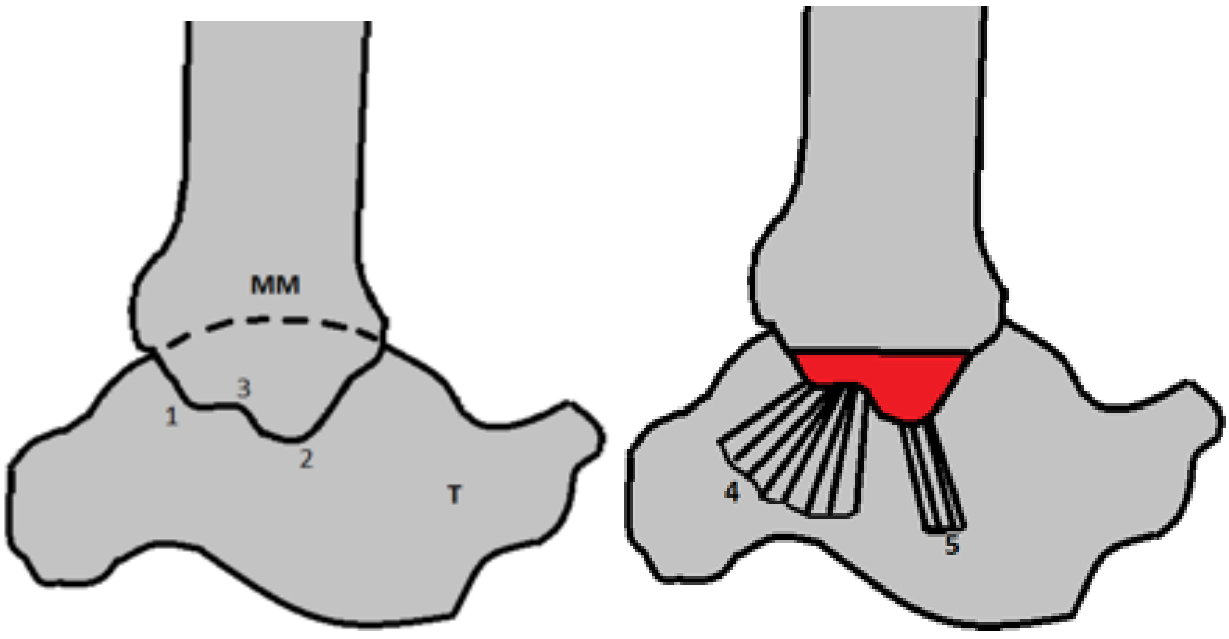

Fig. 1. The fracture line in the medial malleolus was examined by the preoperative CT images. The fractures involving the region associated with anterior and posterior colliculus (including red region) were not included in the study. (1-posterior colliculus, 2- anterior colliculus, 3-intercollicular groove, 4-deep posterior tibiotalar ligament, 5-deep anterior tibiotalar ligament, T = talus, MM = medial malleolus).

Table 1. Comparison of preoperative and postoperative values of tibiofibular congruence parameters

	Preoperative		Postoperative		
	Mean ± SD	**M [Q1-Q3]**	**Mean ± SD**	**M [Q1-Q3]**	***p value***
TCS	6.05 ± 1.85	6 [5-6]	4.19 ± 1.85	4 [4-4]	**< 0.001**
TFO	5.03 ± 2.93	6 [3-7]	8.62 ± 1.04	9 [8-9]	**< 0.001**

M = median, Q = Quartile, SD = standard deviation, TFO = tibiofibular overlap, TCS = tibiofibular clear space

(21.6%) cases, falling in 9 (24.3%) cases, and falling from height in 3 (8.2%) down cases. According to the Denis-Weber classification, 31 (82.8%) cases were classified as type B and 6 (16.2%) cases were classified as type C. Tibiofibular incongruence was determined in 25 (67.6%) cases in the external rotation test after the fixation of both malleolus. In all of these cases, syndesmosis repair was made by crossing 1 syndesmotic screw over the fibula plate. The medial malleolar fracture was fixed with a screw in 28 cases and with tension band technique in 9 cases. The preoperative TCS was measured as 6.05 ± 1.86 mm, postoperative TCS as 4.19 ± 0.40 mm, preoperative TFO as 5.03 ± 2.93 mm, postoperative TFO as 8.62 ± 1.04 mm and postoperative MCS as 4.11 ± 0.57 mm. Postoperative medial clear space of 5 mm and higher was determined in 7 (18.9%) cases. The difference between preoperative and postoperative tibiofibular congruence measurement results was statistically significant (Table 1). The average union time of both malleolus was 7.73 ± 1.61 weeks.

There was no statistical relationship between the fracture type according to the Denis-Weber classification or preoperative syndesmosis dysfunction and postoperative medial clear space congruence ($p > 0.05$) (Table 2).

DISCUSSION

The sequence of injury in supination-external rotation fractures is anterior tibiofibular ligament disruption (SER-I), oblique fibula fracture (SER-II), posterior tibiofibular ligament or posterior malleolar fracture (SER-III) followed by deltoid ligament rupture or medial malleolar fracture (SER-IV) [1]. In other words; according to Lauge-Hansen classification, if a bimalleolar fracture has occurred in SER type 4 injury, the deltoid ligament should be intact. But, there are studies suggesting that in bimalleolar fractures, a medial malleolar fracture may be concomitantly accompanied by deltoid ligament damage. However, these studies suggested that the deltoid ligament is not damaged in fractures of the supracollicular region. Even, this injury was associated with anterior collicular fractures [7, 8]. Bozscyk *et al*. [9] suggested that many fibular fractures traditionally recognized as supination injuries actually can be caused by a pronation mechanism. They stated that the combination of a posterior malleolar fracture and an anterior colliculus fracture of the medial malleolus is indicative of the pronation mechanism [9]. In our study, there were radiographic deltoid insufficiency findings in 18.9% of the bimalleolar fractures with supracollicular medial malleolar fractures. Then, SER

Table 2. Relationship between preoperative syndesmosis dysfunction and Denis-Weber classification and postoperative medial clear space

	Status of preoperative syndesmotic joint		*p* value
	Impaired (n = 25)	**Intact (n = 12)**	
Postoperative medial clear space	4.12 ± 0.67	4.08 ± 0.29	0.857
	Denis-Weber		
	Type B (n = 31)	**Type C (n = 6)**	
Postoperative medial clear space	4.16 ± 0.45	3.83 ± 0.98	0.615

Data are shown as mean ± standard deviation.

type bimalleolar fractures with anterior colliculus fractures in the literature may be a result of the inability of the Lague-Hansen classification system to describe the mechanism of fracture. On the other hand, bimalleolar fractures, even though large medial malleolar fragment fracture, may be accompanied by deltoid ligament damage.

Lauge-Hansen proposed a fracture classification system that is commonly used to evaluate ankle fractures. Based on early experiments on manually created fractures in cadaver ankles, Lauge-Hansen reported that characteristic fracture models appear repeatedly depending on both the position of ankle and the direction of deforming force [11]. The critical review of the study by Lauge-Hansen revealed the lack of a controlled deforming force (since it is applied manually), the lack of information on the position of ankle (such as plantar flexion versus neutral position or dorsiflexion versus neutral position) and uncertainties regarding additional lateral bearing during the test [12]. The deep deltoid component is the strongest part of the ligament and functions as a resistance against external rotation of the ankle when the foot in dorsiflexion [7]. We think that the emergence of findings in our study may be a result of the inability of the Lauge-Hansen system to describe the position of the foot in sagital plane (flexion, neutral or extension). For example, the deep deltoid ligament is expected to be stretched with an ankle in dorsiflexion. The external rotation (ER) force applied to the talus may also damage to the deltoid ligament. If the force applied by the continuing talus rotation to the ankle mortise is forcible, it may contribute to the mechanism of medial malleolar fracture.

Tornetta [7] found that 26% of cases with bimalleolar fracture had deltoid insufficiency based on the evaluation in the peroperative stress test. And, this was associated with a small medial malleolar fragment fracture, and it was suggested that supracollicular fractures had no deltoid ligament injury [7]. Fukuyama *et al*. [8] evaluated the deltoid damage using MR images based on the same study. They reported that the deltoid ligament was injured in 50% of cases with SER bimalleolar fractures, and there was a relationship between small fragments associated with anterior colliculus and deltoid ligament injury. There was no difference in the size of fragment between the medial malleolar fractures with fully ruptured deltoid and those with intact deltoid [8]. However, in this study, the evaluation was made only based on MR findings. It gave no information about instability findings in stress radiographs. We believe that the fact that deltoid ligament damage was less commonly seen in our study might be caused by the inclusion of only supracollicular fractures in the study and/or the ability to display soft tissue structures that did not show radiologic instability finding in MR.

In our clinic, the instability check has been routinely performed with external rotation test under fluoroscopy during peroperative evaluation. Although the deltoid ligament was not intervened in any way during the peroperative period, 18.9% of the cases had a high MCS in the postoperative period. We think that this is associated with the insufficiency of the Lauge-Hansen classification system to describe medial ligament injury. Since when a surgeon who relies on the Lauge-Hansen classification evaluates the peroperative stress radiography after fixing the bimalleolar fracture, the MCS should be routinely normal in this patient according to the Lauge-Hansen classification, hence this might be the result of the fact that he/she failed to evaluate the MCS or to pay attention as required when evaluating the MCS.

Manual external rotation or gravity stress tests are considered as gold standard in the radiographic diagnosis of ankle instability [13]. In Stage 4 injuries, some of cases with medial injuries that do not require surgical treatment may be diagnosed with instability in external rotation or gravity stress tests [14]. Some recent studies reported that weight-bearing radiograph is a physiologic test of ankle stability, simulating the loads which the patient will put on the joint in the joint and ligament recovery period [14, 15]. We also used weight-bearing radiographs in our patients. They are still gold standard stress tests in decision-making for ankle instability. This may be considered as one of the limitations of our study. On the other hand, in the case of abnormal medial clear spaces that can be detected even in weight-bearing radiographs, these may be expected to increase if stress tests are performed. Therefore, we believe that our study could provide sufficient evidence on radiographic findings of deltoid ligament injury which may be associated with SER-4 bimalleolar fractures.

The fact that the cases with high MCS were not validated by MR images may be considered as a lim-

itation of our study. Because there may be no correlation between stress radiographs and soft tissue injuries detected by MR imaging [5, 13]. On the other hand, MR may probably cause overestimation of complete ligament ruptures in cases with ankle fracture [4]. Also, magnetic resonance imaging may be not an ideal test for acute ankle fractures. Firstly, there is no clinical study suggesting that MRI includes diagnostic test performance statistics (sensitivity, specificity) to detect the degree of deltoid ligament injury [14].

Radiographic evaluation of MCS abnormal increase is an indirect method ısed in defining deltoid ligament injury [2]. Therefore, MCS alone may be insufficient to define deltoid ligament injury [13, 16, 17]. In our study, no deltoid ligament repair was performed in any case we detected an increase in medial clear space during the follow-up period. We also did not have records of functional and pain scores.

Limitations

Limitation of our study is that the correlation between MCS values we detected and deltoid ligament injury could not be demonstrated with more specific methods and the effect of this condition on clinical and functional outcomes was unknown. Other limitations of our study may include its retrospective nature, the low number of cases, and the lack of our knowledge on functional results. Because proving the clinical reflection of the significant radiologic findings obtained will add value to the study. This can be revealed only by a prospective, randomize-controlled study that has a long-term follow-up and a sufficient number of cases, and includes functional and clinical symptoms.

CONCLUSION

In SER type 4 bimalleolar fractures with a large medial malleolar fragment fracture, weight-bearing radiographs may show an increase in MCS. This means that a medial malleolar fracture in bimalleolar fractures may be also accompanied by deltoid ligament injury. The Lauge-Hansen classification system may be insufficient to identify a medial ligament injury. In SER Type 4 bimalleolar fractures, peroperative MCS should be evaluated, and the presence of instability findings should be warning to the surgeon in terms of deltoid ligament injury.

Authors' Contribution

Study Conception: AY, MY, TOB, BK, Yİ, SSD, MÇK, HG; Study Design: AY, MY, TOB, BK, Yİ, SSD, MÇK, HG; Supervision: AY, MY, TOB, BK, Yİ, SSD, MÇK, HG; Funding: AY, MY, TOB, BK; Materials: AY, MY, TOB, BK; Data Collection and/or Processing: AY, MY, Yİ, SSD, MÇK; Statistical Analysis and/or Data Interpretation: AY, MY, TOB, BK, Yİ, SSD, MÇK, HG; Literature Review: AY, MY, TOB, BK, Yİ, SSD, MÇK, HG; Manuscript Preparation: AY, MY, TOB, BK, Yİ, SSD, MÇK, HG and Critical Review: AY, MY, TOB, BK, Yİ, SSD, MÇK, HG.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2021;7(5):472-480 Original Article

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Comparison of contrast-enhanced magnetic resonance angiography and digital subtraction angiography in the evaluation of renal artery stenosis and detecting of accessory and polar arteries

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ABSTRACT

Objectives: Renal artery stenosis (RAS) is the most common cause of secondary hypertension. RAS may cause renal insufficiency, uncontrolled hypertension and is associated with increased cardiovascular morbidity and mortality. We aimed to evaluate the accuracy of contrast enhanced Flash 3D Renal Magnetic Resonance Angiography (MRA) in the depiction of the RAS also detecting of accessory and polar renal arteries with intraarterial digital subtraction angiography (DSA) still serving as the reference standard.

Methods: In this retrospective case-control study, we reviewed contrast enhanced Flash 3D Renal MRA and DSA of 71 patients who were suspected of having RAS and underwent DSA after MRA within 15 days. DSA was accepted as gold standart and the specificity, sensitivity and accuracy of MRA were determined.

Results: Overall sensitivity and specificity values of contrast enhanced Flash 3D Renal MRA in detecting stenosis were 96.1% and 76.3% respectively.

Conclusions: Contrast enhanced Flash 3D Renal MRA is a reliable noninvasive imaging modality in the diagnosis of RAS.

Keywords: Renal artery stenoses, magnetic resonance angiography, digital substraction angiography

Renal artery stenosis (RAS) is one of the important and treatable causes of hypertension and end-stage renal failure [1]. In 90% of the cases, the cause of stenosis is atherosclerosis. Other important causes are fibromuscular dysplasia and vasculitis such as Takayasu arteritis and polyarteritis nodosa. In 12-14% of dialysis patients and 1-5% of hypertensive patients, the underlying cause is atherosclerotic renal artery stenosis. It is found at increasing rates as in 15% of persistent hypertension, 20% of coronary heart disease, and 30-40% of peripheral artery disease cases [2-4].

Even if the high blood pressure is reduced with medication, not correcting the renal artery stenosis may cause a decrease in renal blood flow and ischemic damage [5, 6]. Therefore, it is important to detect renovascular hypertension before it causes renal dysfunction [7]. Treatment of renal artery stenosis by the percutaneous transluminal route or surgery facilitates the control of high blood pressure and preservation of kidney function [8-10].

The morphological imaging methods used in the diagnosis of RAS are Doppler ultrasonography, computed tomography (CT), magnetic resonance (MR) an-

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giography and conventional angiography.

Digital Subtraction Angiography (DSA) is considered the gold standard in the diagnosis of renal artery stenosis. The fact that it is an invasive method restricts its use for screening. In addition, diagnostic angiography is performed first in each patient planned to undergo endovascular treatment for renal artery stenosis. The advantages of DSA are the high resolution, its capacity to detect stenosis in the branches of the renal artery the hemodynamic significance of the stenosis by measuring the trans stenotic pressure difference [11].

Magnetic Resonance Angiography (MRA) is an imaging method that shows the vascular anatomy in detail and eliminates the risks of iodinated contrast and ionizing radiation. Contrast-mediated MRA is an effective method used in the diagnosis of renal artery stenosis with its advantages such as high resolution, ability to show the anatomy of the renal artery and the distal segmental branches, and its applicability in a short time (20-40 sec) [12, 13].

METHODS

Patients who had undergone renal MRA examination in the last five years were scanned in our archive. Out of 631 tests, those performed for preparation prior to renal transplantation, patients who had previously undergone endovascular treatment (patients with stents) and patients who had not undergone a DSA examination within 15 days of the MRA test, were excluded from the study. Among the remaining patients, the images of 75 patients who had undergone DSA within 15 days of the MRA examination were re-evaluated. Four patients whose images were not diagnostic were excluded from the study. The images of 71 patients who met the criteria were analyzed retrospectively.

A total of 71 (24 male 33%, 47 female 67%) patients between the ages of 18 and 84 (average 63.6) were included in the study. First, contrast enhanced renal MRA and then, bilateral selective renal DSA within 15 days were performed in all patients. The MRA and DSA images were evaluated by two separate radiologists.

MR Angiography Protocol

All patients underwent a contrast-enhanced Flash (fast low angle shot) 3D coronal T1 weighed MR sequence in a 1.5 Tesla MR (Magnetom Symphony; Siemens Medical Systems, Erlangen, Germany) unit with the breath-hold “Care bolus” technique. Peripheral vascular access was established from the level of the antecubital fossa, preferably in the right upper extremity, with a 22-gauge needle. Body bandage was used since the abdominal aorta and renal arteries would be scanned. The body bandage was placed on the patient with the upper end at the level of the nipple and the lower end at the level of the umbilicus.

Patients were placed in the magnet in the headfirst position. Scout images were obtained. Before administration of the intravenous contrast agent, patients were told to hold their breaths and non-contrast masked images were obtained. Then, an infusion of 0.02 mmol/kg contrast agent (Gadobenate Dimeglumin, Multihance; Bracco SpA, Milan, Italy) was started. Using the "Care Bolus" technique, the moment the contrast agent appeared on the screen, the sequence was begun, and images were obtained. Immediately after completion of contrast agent administration, 15 ml serum saline was pushed at a rate of 0.5 mL/seconds, allowing the entire contrast medium to pass into the body. The coronal Flash 3D T1 weighted sequence was then repeated 2 times with a short breath break. The examination parameters have been summarized in the table (Table 1).

Intraarterial DSA Protocol

Catheter angiography was performed using the digital subtraction technique (Multistar; Siemens, Erlangen, Germany). After covering of the patient in ac-

Table 1. Parameters of contrast-enhanced Flash 3D coronal T1 weighed renal MRA sequence

TR (time of repetetation)	3.64 msn
TE (time of echo)	1.36 msn
FOV (field of view)	420 mm
Slice thickness	2 mm
FA (flip angle)	25 degree
Resolution	40×512
Time	18 sec
NEX	1
SLAP	1

cordance with the sterilization rules and local anesthesia with lidocaine, a 5 French (Fr) vascular sheath was inserted into the main femoral artery from the right/left main femoral artery using the Seldinger method and aortography images were obtained after advancing the pigtail catheter into the distal abdominal aorta. Selective bilateral renal angiography images were obtained by selectively entering the main renal arteries with a shepherd hook catheter. Angiography images were obtained by selectively entering accessory and polar arteries with the shepherd hook catheter, which were observed in the evaluation of the aortography images. If a non-enhanced area were detected in the renal parenchyma during the selective injections from the main renal artery, the areas suspected in aortography were scanned with a shepherd hook catheter.

Evaluation of the Images

The MRA and DSA images were evaluated by two separate radiologists. MIP images and raw images were used in the evaluation of the MRA images. For the suspicious spots on MIP images, the raw images were evaluated in 3D to make decisions.

While examining the images, the main purpose was to determine the presence and the degree of renal artery stenosis. In addition, imaging of accessory and polar arteries and detection of pathological diseases in the renal artery such as FMD were also considered as other purposes of the study.

In our study, renal artery stenosis was divided into 4 set:

Grade 0: No renal artery stenosis, normal renal artery.

Grade 1: <50% stenosis

Grade 2: Stenosis of 50% and higher

Grade 3: Total occlusion of the renal artery

In our study, stenoses of 50% and higher were regarded as significant stenosis, because they are candidates for DSA and endovascular treatment. Similar rating tables are available in the literature. In his study, Fleischmann described the stenoses between 50-70% as significant and stenoses higher than 70% as severe stenosis.

Statistical Analysis

Analysis of the data was carried out using the SPSS 11.5 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) package program. The Spearman's Rho test was used to determine whether there was a significant difference between DSA and MRA staging in terms of diagnosis. To assess the diagnostic performance of MRA compared to DSA, the

Table 2. DSA results of 142 main renal arteries

DSA		Male n (%)	Female n (%)	Total n (%)
Grade of* stenosis				
Right Main Renal Arteries	0	3 (12.50)	17 (36.17)	20 (28.17)
	1	7 (29.17)	15 (31.91)	22 (30.99)
	2	12 (50.00)	13 (27.66)	25 (35.21)
	3	2 (8.33)	2 (4.26)	4 (5.63)
Left Main Renal Arteries	0	5 (20.83)	13 (27.66)	18 (25.35)
	1	11 (45.83)	13 (27.66)	24 (33.80)
	2	8 (33.33)	20 (42.55)	28 (39.44)
	3	0 (0)	1 (2.13)	1 (1.41)
Existensce of Accessory Artery	No	21 (87.50)	44 (93.62)	65 (91.55)
	Yes	3 (12.50)	3 (6.38)	6 (8.45)
Existensce of Polar Artery	No	22 (91.67)	41 (87.23)	63 (88.73)
	Yes	2 (8.33)	6 (12.77)	8 (11.27)

*Grade 0 = No stenosis, Grade 1 = <%50 stenosis, Grade 2 =>%50 stenosis, Grade 3 = Total occlusion. Grade of stenosis showed as number of patients and percentage in gender groups. In 71 patients existence of polar and accessory arteries showed as number of patients and percentage in gender groups.

sensitivity, specificity and the diagnostic accuracy rates were calculated. The significance of diagnostic concordance between DSA and MRA was calculated with the correlation coefficient. The results were considered statistically significant for p values of < 0.05.

RESULTS

In 71 patients, a total of 142 main renal arteries were examined, right and left separately. While 38 of the main renal arteries examined in DSA were normal (grade 0), 46 had less than 50% (grade 1), 53 of the major renal arteries had 50% or higher stenosis (grade 2), and 5 major renal arteries were totally occluded (grade 3). Six accessory arteries and eight polar arteries were detected (Table 2). Based on DSA, 58 patients had stenosis that caused significant hemodynamic changes (grades 2 and 3).

In the evaluation of the same patients with MRA, 33 of the major renal arteries examined were normal (grade 0), 39 had less than 50% (grade 1), 64 major renal arteries had 50% or higher stenosis (grade 2) and 6 major renal arteries were found to be totally occluded (grade 3). On MRA, 9 accessory arteries and 5 polar arteries were detected.

Based on MRA, a total of 70 patients had stenosis causing significant hemodynamic changes (grade 2 and 3). In the correlation with DSA, it was concluded that one major renal artery was incorrectly evaluated as total occlusion and 11 major renal arteries were incorrectly evaluated within grade 2 (> 50% stenosis) (Table 3).

In the comparison of diagnostic concordance of the DSA and MRA in all grades of stenosis (142 major renal arteries); the Spearman's Rho was calculated as 0.862 and $p < 0.01$. The diagnostic concordance of DSA and MRA was found to be significant and well correlated (Fig. 1). The sensitivity of MRA in the detection of all renal artery stenoses was calculated as 96.1% and specificity as 76.3%.

In the comparison of diagnostic concordance of the DSA and MRA in grade 2 and 3 stenoses (70 major renal arteries); Spearman's Rho was calculated as 0.885 and $p < 0.01$. It was shown that the diagnostic concordance of DSA and MRA was significant and well correlated (Fig. 2).

While the number of accessory arteries was 6 on DSA, 9 accessory arteries were observed on MRA. The polar artery number was found to be 8 on DSA. Five of these polar arteries were detected on MRA. The main renal artery showing early segmentation, double renal artery originating from a common root, thin polar artery or its origin being localized inferiorly

Table 3. MRA results of 142 main renal arteries.

MRA		Male n (%)	Female n (%)	Total n (%)
Grade of* stenosis				
Right Main Renal Arteries	0	3 (12.50)	13 (27.66)	16 (22.54)
	1	6 (25.00)	13 (27.66)	19 (26.76)
	2	12 (50.00)	19 (40.43)	31 (43.66)
	3	3 (12.50)	2 (4.26)	5 (7.04)
Left Main Renal Arteries	0	5 (20.83)	12 (25.53)	17 (23.94)
	1	9 (37.50)	11 (23.40)	20 (28.17)
	2	10 (41.67)	23 (48.94)	33 (46.48)
	3	0 (0)	1 (2.13)	1 (1.41)
Existensce of Accessory Artery	No	19 (79.17)	43 (91.49)	62 (87.32)
	Yes	5 (20.83)	4 (8.51)	9 (12.68)
Existensce of Polar Artery	No	22 (91.67)	44 (93.62)	66 (92.96)
	Yes	2 (8.33)	3 (6.38)	5 (7.04)

Grade of stenosis showed as number of patients and percentage in gender groups. In 71 patients existence of polar and accessory arteries showed as number of patients and percentage in gender groups.

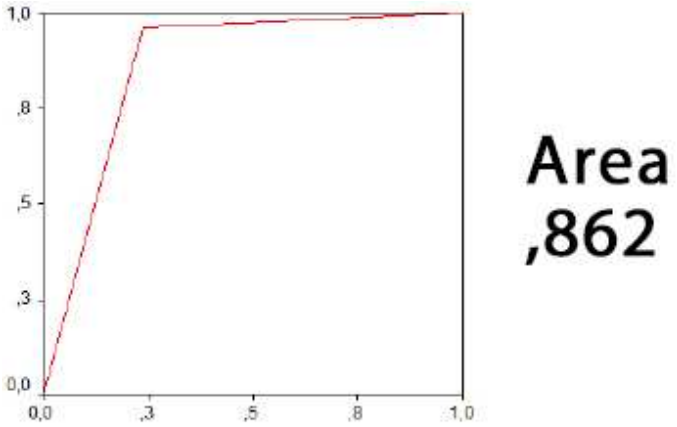

Fig. 1. In the comparison of diagnostic concordance of the DSA and MRA in all grades of stenosis; the Spearman's Rho test was showed as ROC curve graphic and the area was calculated 0.862.

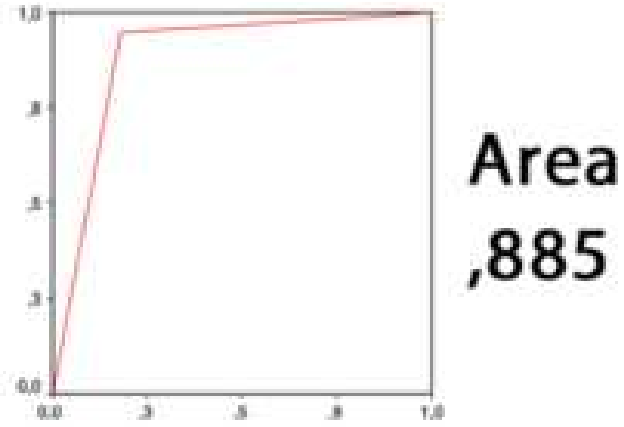

Fig. 2. In the comparison of diagnostic concordance of the DSA and MRA in grade 2 and grade 3 stenosis; the Spearman's Rho test was showed as ROC curve graphic and the area was calculated 0.885.

can be listed among the reasons of these discordances.

The Spearman's Rho test was used to examine the diagnostic performance of MRA compared to DSA in the detection of accessory arteries. The correlation coefficient 0.797 shows that a correlation is present between the two tests ($p < 0.01$).

Although the diagnostic performance of MRA decreases in the detection of polar arteries, a correlation is present between the two tests, albeit low (Spearman's Rho 0.598 and $p < 0.01$).

DISCUSSION

DSA is the gold standard method in the evaluation of renal arteries, but a non-invasive, reliable imaging method is needed due to complications caused by arterial catheterization [14].

In the detection of renal artery stenoses in our study, contrast-enhanced Flash 3D MR angiography demonstrated a good correlation with DSA with values of 96.1% and specificity of 76.3% and was deemed successful in terms of diagnostic concordance (Spearman's Rho 0.86) and no statistical difference was detected in terms of diagnostic success when compared to DSA ($p < 0.01$). The findings are consistent with the literature [12, 15, 16]. Recent concerns about the association between gadolinium-based contrast agents and nephrogenic systemic fibrosis has initiated the search for reliable non-enhanced renal MRA techniques [17]. Non-contrast-enhanced MRA techniques are successful in detecting the presence of renal artery stenosis, but in the evaluation of renal artery stenosis of 50% and higher in particular, it has a tendency to overestimate stenosis. Some studies have recommended to perform the first examination without contrast if renal artery stenosis is suspected, and to continue the tests with contrast enhanced MRA if needed after the images are evaluated [18]. In our study non-contrast enhanced MRA techniques not included because we have very small number of cases. Today, 3.0 T non-enhanced MRA techniques have been developed, but with these techniques, diagnostic problems such as overestimating stenosis and low sensitivity (80%) and specificity (63%) remain, but on the decrease [19].

Since the day they were first introduced, contrast mediated MRA techniques continue to be updated to achieve higher temporal and spatial resolution. Contrast-enhanced MRA is developing with techniques such as view sharing techniques (TRICKS, TWIST, DISCO, CAPR) that improve speed and parallel acquisition techniques (SENSE, REFS) [20]. These techniques have advantages such as better temporal resolution and dynamic information provision, but spatial resolution is similar to standard contrast MRA techniques [21]. It may be possible to obtain images with higher spatial and temporal resolution with new MR angiography techniques such as 3D through-time radial generalized auto calibrating partially parallel acquisition (GRAPPA). In the literature, there are Renal MR Angiography studies with the GRAPPA technique, but it is not possible to make objective interpretations, since comparative studies with conventional

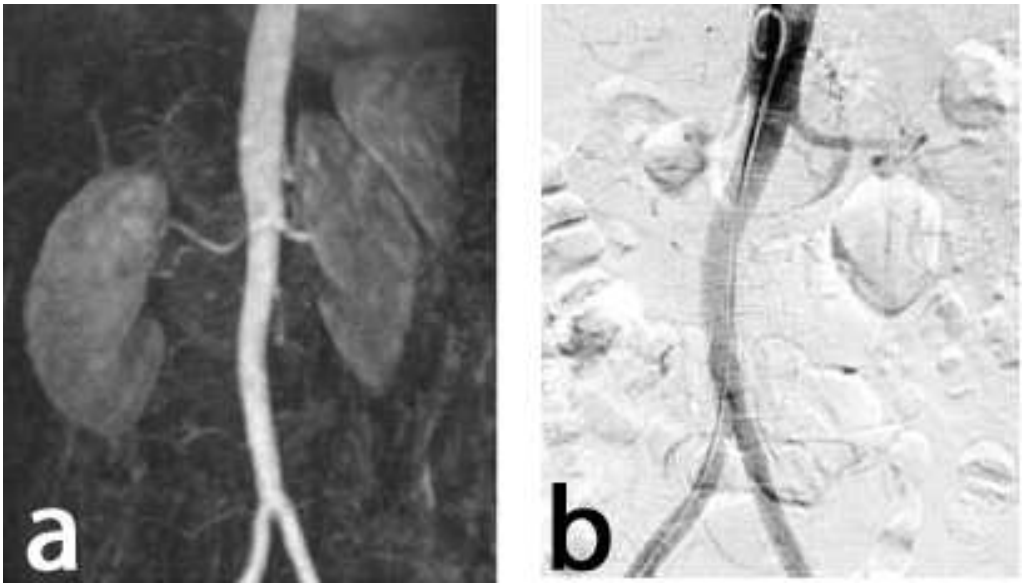

Fig. 3. Contrast enhanced 3D Renal MRA coronal MIP image showed grade 2 stenoses of right main renal artery proximal part (a). Abdominal aortagraphy DSA image showed a severe angulation of the right main renal artery proximal part in the cranio-caudal direction close to the ventral surface of the abdominal aorta, and no evidence of stenosis (b).

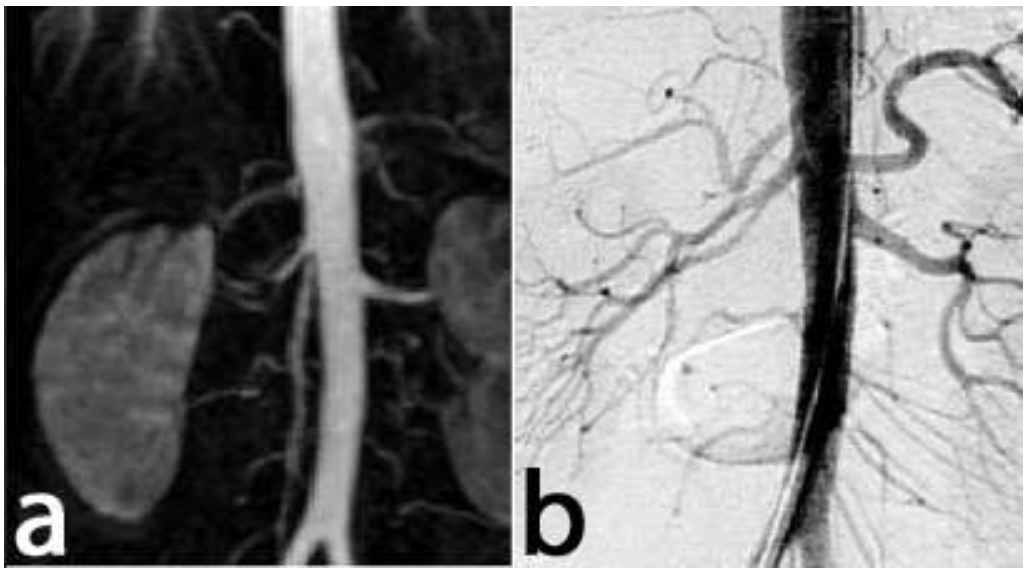

Fig. 4. Contrast enhanced 3D Renal MRA coronal MIP image showed grade 2 stenoses of right main renal artery mid part (a). Abdominal aortagraphy DSA image showed renal artery had early segmentation and no evidence of stenosis (b).

MR sequences or DSA have not been conducted yet [22].

Stenoses above 50% in the renal artery lead to hemodynamic changes. The diagnosis of Grade 2 and above stenoses with MRA is important, because they may need to be treated. In our study, 64 main renal arteries were classified as grade 2 on MR angiography. However, this number stopped at 53 on DSA. Despite not causing meaningful stenosis on DSA, 11 main real arteries were considered to be in the meaningful stenosis group on MRA. Similar to the literature, MR angiography overestimated the stenoses. In the retrospective comparative evaluation of these false positive cases, potential reasons other than the exaggerated appearance of the potential stenosis were identified in 3 cases. A severe cranio-caudal direction angulation in the main renal artery proximal part close to the ventral surface of the abdominal aorta was detected in 2 cases (Fig. 3). One case was diagnosed with grade 2 despite not having any stenosis on DSA in a renal artery that showed early segmentation (Fig. 4). One of the limitations of contrasted MRA is the inadequate evaluation

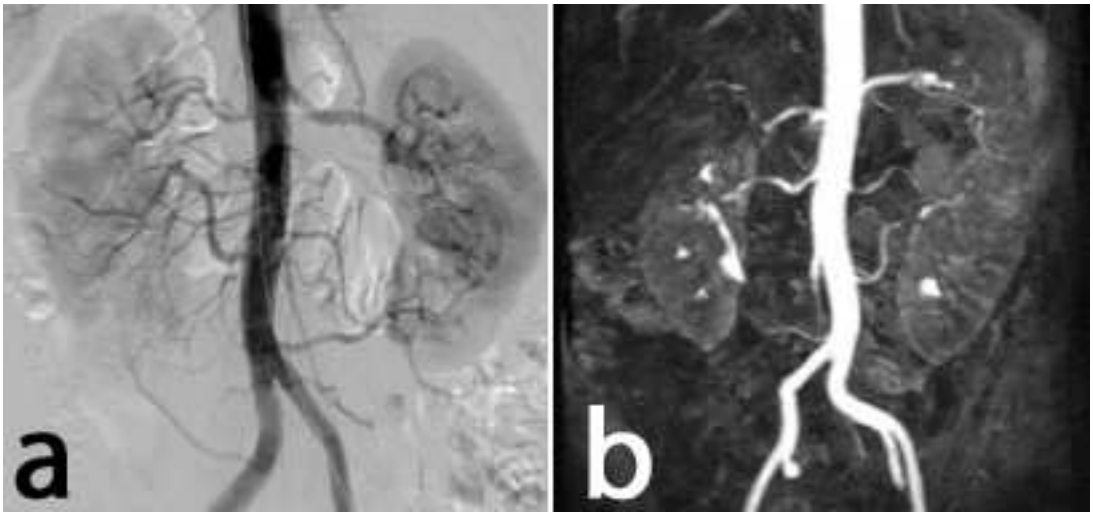

Fig. 5. Abdominal aortagraphy DSA image showed a left polar renal artery arised from left main iliac artery and reached to the left kidney lower pole (a). No evidence of polar artery on contrast enhanced 3D Renal MRA coronal MIP image (b).

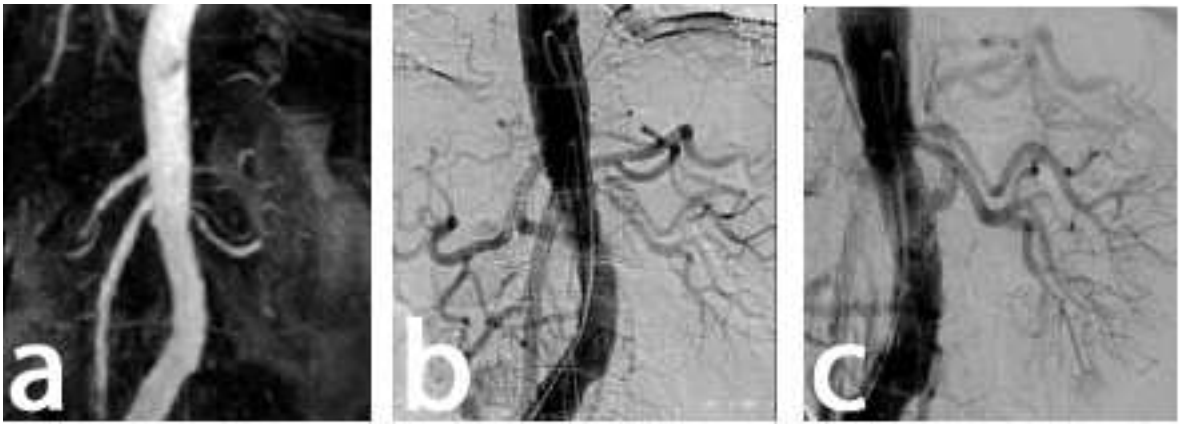

Fig. 6. Contrast enhanced 3D Renal MRA coronal MIP image showed an accessory renal artery arised from aorta and found adjacent to the left main renal artery (a). Abdominal aortagraphy DSA image showed two main renal arteries arised from same origin and both of them had grade 2 stenosis (b). Abdominal aortagraphy DSA image showed two left main renal arteries treated with stent placement (c).

of segmental branches. In addition, although stenosis is not common in the distal 1/3 section of the renal artery, the evaluation of stenoses in this section is problematic on MRA [19, 23].

In our study, 5 renal arteries were detected to be occluded on DSA. On MRA, 6 main renal arteries were regarded as grade 3. One main renal artery, which was observed as grade 2 on DSA but had stenosis higher than 90%, was incorrectly evaluated as stage 3 on MRA. Exaggerated signal loss at stenosis levels can prevent the accurate identification of the degree of stenosis. Although such signal losses on MRA are largely prevented by contrast enhancement, some signal loss occurs in areas with turbulent flow, such as severe stenosis or the orifice level. In particular, slight signal losses that appear as a localized lumen narrowing at the orifice, attention should be paid to the dephasing artifact that causes signal loss [24].

Accessory arteries enter the kidney from the hilum together with the main renal artery, and the polar arteries enter the kidney directly from the capsule outside the hilum. The correct detection of these arteries is very important in the pre-transplant evaluation of living kidney donors. In our study, polar and accessory arteries were detected in 8.4% and 11.2% of the cases, respectively. Retrospectively, cases without a polar artery on MRA were re-evaluated. The first reason for the inability to detect the polar arteries can be the origin of the polar artery originating from the distal abdominal aorta or the main iliac artery far from the renal artery origin or being very thin (Fig. 5).

In our study, the detection rate of accessory arter-

ies was higher compared to polar arteries. The correlation coefficients were 0.797 and 0.598, respectively. While the number of accessory arteries was 6 on DSA, 9 accessory arteries were observed on MRA. When the images, which had been incorrectly evaluated as accessory arteries in MRA, were retrospectively re-evaluated, it was observed that the discordances were due to reasons such as the main renal artery showing early segmentation, and a double renal artery originating from a common root (Fig. 6).

Contrast-enhanced Flash 3D MRA is a reliable non-invasive imaging method with high specificity and sensitivity in the evaluation of renal artery stenosis. Although the diagnostic performance of MRA in the detection of accessory renal arteries and polar arteries decreases compared to the successful rates for stenosis detection, there is no statistically significant difference when compared to DSA.

CONCLUSION

We concluded in our study: severe angulations and early segmentations of renal arteries are weak points of MRA in the evaluation of renal artery stenosis. In our study we found weak points of MRA in the detection polar and accessory arteries: distal origin of the polar artery and early segmentation main renal artery.

Authors' Contribution

Study Conception: CB; Study Design: CB; Supervision: CB; Funding: UMY; Materials: CB, UMY; Data Collection and/or Processing: UMY; Statistical Analysis and/or Data Interpretation: UMY; Literature Review: UMY; Manuscript Preparation: UMY and Critical Review: UMY.

Conflict of interest

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Fragmented QRS formation may be associated with increased carotid intima-media thickness in patients with end-stage renal disease

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ABSTRACT

Objectives: The aim of this study was to evaluate the relationship between carotid intima-media thickness and the presence of fragmented QRS in end-stage renal disease patients.

Methods: The study included 100 end-stage renal disease patients who received hemodialysis treatment. Two groups were formed according to the presence of electrocardiography and fragmented QRS: the fragmented QRS (+) (Group I) and the fragmented QRS (-) (Group II). Echocardiographic measurements of the cardiac dimensions and carotid intima-media thickness were examined.

Results: The study population included 41 end-stage renal disease patients with fragmented QRS (48.8% male) and a control group of 59 end-stage renal disease patients without fragmented QRS (35.6% male) on electrocardiography. Left ventricular end systolic diameters ($p = 0.012$),left ventricular end diastolic diameters ($p < 0.001$), left atrial diameter ($p = 0.001$), interventricular septal thickness ($p < 0.001$), posterior wall thickness ($p < 0.001$) and left ventricular hypertrophy ($p < 0.001$), blood urea nitrogen ($p = 0.011$), creatinine ($p = 0.014$), uric acid ($p < 0.05$) and parathyroid hormone ($p < 0.05$) values were found to be significantly increased in the fragmented QRS (+) group. The right and left carotid intima-media thickness values were significantly higher in fragmented QRS (+) patients than fragmented QRS (-) patients (right:[0.81 ± 0.19] vs [0.62 ± 0.14] mm, $p < 0.001$) and left: [0.83 ± 0.19 vs 64 ± 0.14], $p < 0.001$; respectively).

Conclusions: In end-stage renal disease patients, the presence of fragmented QRS, left ventricular hypertrophy and increased carotid intima-media thickness are important markers in the evaluation of the inflammatory process of atherosclerosis.

Keywords: fQRS, end-stage renal disease, carotid intima-media thickness, left ventricular hypertrophy

End-stage renal disease (ESRD) is an independent risk factor for cardiovascular (CV) complications such as sudden cardiac death, arrhythmias and heart failure. Impaired renal function causes increased inflammation and coagulation, anemia, left ventricular hypertrophy, endothelial dysfunction, and arterial calcification [1, 2]. ESRD patients have been reported to experience 2-fold more CV events than the general

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population [3]. Carotid intima-media thickness (cIMT) is a non-invasive marker that is known to be associated with the presence of coronary artery disease and stroke [4, 5] and is an independent predictor of CV morbidity and mortality in ESRD patients. It has been reported that cIMT can strongly predict future CV events and mortality in chronic renal disease (CKD) patients [6]. Endothelial dysfunction and subsequent atherosclerosis are held responsible for increased intima thickness while increased media thickness results from smooth muscle hypertrophy due to hypertension. Carotid arteries are the most frequently used vessels because of the superficial location, ease of visualization, size and immobility [7].

Fragmented QRS (fQRS) complexes are a depolarization abnormality and fQRS is a marker of myocardial fibrosis or scar tissue [8, 9]. It is shown that LV diastolic dysfunction in hemodialysis patients with fQRS is more severe than in patients without fQRS, and fQRS is associated with systolic and diastolic dysfunction of the left ventricle in kidney transplant patients. It was noted that fQRS can be used for risk classification in hemodialysis (HD) patients [10].

The aim of this study was to evaluate the relationship between cIMT and the presence of fQRS in ESRD patients.

METHODS

This study included 100 ESRD patients who had been receiving HD treatment three times a week for at least six months. Routine 12-lead ECGs were obtained in the patients who were then separated into two groups as the fQRS (+) group (Group I) and the fQRS (-) group (Group II). cIMT measurements were taken of all the patients. Demographic characteristics, comorbidities and medication use were recorded and routine biochemical tests were performed. The study protocol was approved by the Erciyes University, Kayseri. Local Ethics Committee of the hospital in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines and written informed consent was obtained from all participants. Patients with acute coronary artery syndrome, rheumatic heart disease, cardiomyopathy, pregnancy, or atrial fibrillation were not included in the study. ECGs with pace rhythm, typical bundle branch block or any kind of significant conduction abnormality were also excluded from the study.

Biochemical Assessment

Blood samples were collected in the morning of a weekday from an antecubital vein after 12-hours of fasting without dialysis. Routine serum biomarkers were examined, including sodium (Na), potassium (K), calcium (Ca), phosphorus, glucose, blood urea nitrogen (BUN), creatinine (Cr), uric acid, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglyceride (TG), albumin, total protein, alkaline phosphatase, parathormone (PTH), ferritin, and high sensitive c-reactive protein (hsCRP). Values were calculated using standard laboratory methods (Beckmann Coulter aU5800 Autoanalyser, Beckmann Coulter Inc, Brea, California).

Detection and Definition of fQRS

Standard 12-lead electrocardiograms (ECGs) were obtained from all patients on a Nihon Kohden Cardio-

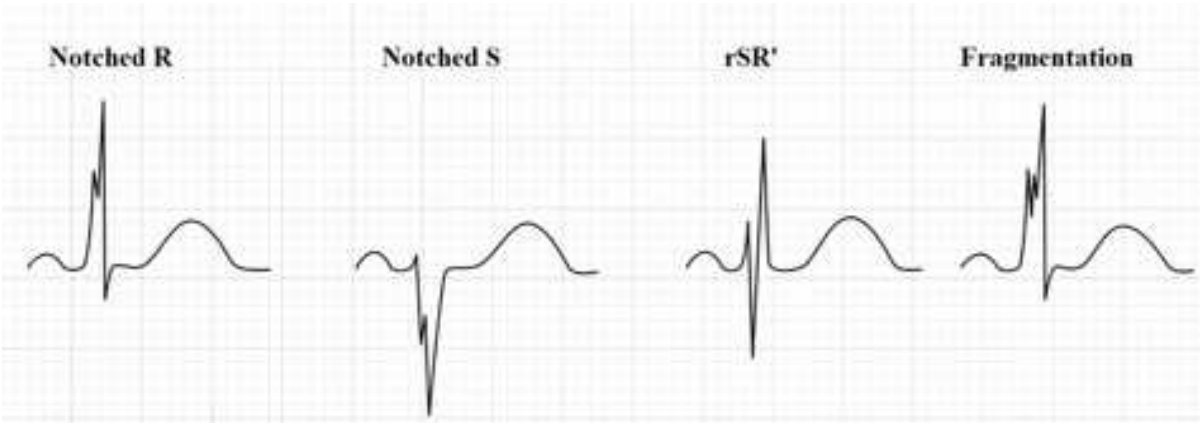

Fig. 1. Fragmented QRS examples.

fax ECG-9132 device with a paper-speed of 25 mm/s, amplitude of 10 mm/mv, and a filter range of 0.15 to 100 Hz. fQRS was defined as the presence of an additional R wave (R'), notching of the R or S wave, or the presence of fragmentation (more than one R') in two contiguous leads (Fig. 1) [11]. Typical right or left bundle branch block pattern (QRS ≥ 120 ms) and incomplete right bundle branch block were excluded. The ECGs were analyzed by 2 independent, experienced cardiologists, who were blinded to all data.

Transthoracic echocardiography was applied to all the patients by two cardiologists using a Vivid 5 echocardiography device (GE Vingmed Ultrasound AS, Horten, Norway), and 3.2 mHz adult prob. Echocardiographic measurements of the cardiac dimensions were taken using parasternal short axis scanning with the patient in the left supine position, according to the American Society of Echocardiography guidelines [12]. Basic measurements included left ventricular end diastolic and end systolic diameters, left ventricular posterior wall thickness (PWT) interventricular septal thickness (IVST), end-diastolic and end-systolic ventricular diameters (LVEDD and LVESD) and left atrial diameter (LAD). Left ventricular ejection fraction (LVEF) was measured using the biplane Simpson and Teicholz method [13].

Measurement of Carotid Intima-Media Thickness

Carotid IMT measurements were taken with the patient in the supine position. Ultrasonography examinations were performed by two cardiologists. (Philips Ultrasound, Bothell, WA 98021 USA, L18-5 vascular probe). The morphology of both arterial carotid arteries, internal carotid artery and carotid bulb were examined in detail. The presence of atherosclerotic plaque and IMT were evaluated separately. Only the posterior (remote) wall was evaluated, and the IMT was measured in the area 1 cm proximal of the carotid bifurcation. Three different scanning angles were used: anterior oblique, lateral, and posterior oblique. The average value of the measurements obtained at these angles was calculated. For ultrasound analysis, the intima-media thickness was measured via the characteristic echogenicity of the lumen-intima and media-adventitia surfaces.

Statistical Analysis

Data obtained in the study were analyzed statistically using IBM SPSS Statistics for Windows, Version 19.0. software (IBM Corp. Armonk, NY, USA). Conformity of the variables to normal distribution was examined using analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk test). Descriptive statistics were reported as mean ± standard deviation (SD) values for continuous variables with normal distribution and as frequency (n) and percentage (%) for categorical variables. Continuous variables were compared between groups using the Student's t-test when data distribution was normal. Comparisons of categorical variables were made using the χ^2 test. A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

A total of 100 patients (59 % female, median age 55.19 ± 13 years) were included in the study. The study population included 41 ESRD patients with fQRS (48.8% male) on ECG and a control group of 59 ESRD patients without fQRS (35.6% male). The demographic characteristics, medications and comorbidities of both groups are shown in Table 1. Age, gender, body mass index (BMI), duration of hemodialysis (the time since the first entry into hemodialysis), diabetes mellitus, hypertension, smoking status and medications were similar in the two groups. The laboratory test results and echocardiographic findings of the ESRD patients with and without fQRS are presented in Table 2. LVESD ($p = 0.012$), LVEDD ($p < 0.001$), LAD ($p = 0.001$), IVST ($p < 0.001$), PWT ($p < 0.001$) and left ventricular hypertrophy (LVH) ($p < 0.001$) were found to be significantly increased in the fQRS (+) group (Table 2). Serum biomarkers of BUN ($p = 0.011$), creatinine ($p = 0.014$), uric acid ($p < 0.05$) and PTH ($p < 0.05$) were found to be significantly increased in the fQRS (+) group. The right and left cIMT values were significantly higher in fQRS (+) patients than in fQRS (−) patients (right: [0.81 ± 0.19] vs [0.62 ± 0.14] mm, $p < 0.001$) and left: [0.83 ± 0.19 vs 64 ± 0.14], $p < 0.001$; respectively).

DISCUSSION

The results of this study demonstrated that the

Table 1. The demographic characteristics, medications, laboratory tests and co-morbidities of the study population

	fQRS (+) Group (n = 41)	fQRS (-) Group (n = 59)	*p* value
Age (years)	56.5 ± 12.3	55.1 ± 14.4	0.632
Gender (M/F)	20/21	21/38	0.187
BMI (kg/m²)	27.2 ± 4.8	25.9 ± 4.6	0.205
Smoker (n, %)	12.2	8.5	0.542
Diabetes Mellitus (%)	29.3	27.1	0.814
Hypertension (%)	80.5	72.9	0.381
Duration of hemodialysis (month)	78.2 ± 62.3	65.6 ± 52.3	0.279
CAD (%)	70.7	57.6	0.182
CVD (%)	2.4	5.1	0.642
PAD (%)	9.8	11.9	0.740
BUN (g/dL)	57.3 ± 15.8	49.2 ± 15.3	**0.011**
Creatinine (g/dL)	7.4±2	6.5±1.5	**0.014**
GFR (mL/min)	8 ± 2.5	8.3 ± 1.9	0.428
Uric Acid (mg/dL)	5.2±1.5	4.6 ± 1.3	**0.041**
Ca (mg/dL)	8.9 ± 0.9	9 ± 0.7	0.617
Phosphorus (mg/dL)	4.6 ± 1	4.6 ± 1.4	0.795
PTH (pg/mL)	510 ± 467	354 ± 250	**0.033**
Total Protein (gr/dL)	7.2 ± 0.6	7.4 ± 0.7	0.254
Albumin (g/dL)	4 ± 0.3	4.1 ± 0.46	0.550
Total Cholesterol (mg/dL)	107 ± 38	189 ± 46	0.751
Glucose (mg/dL)	107 ± 38	120 ± 67	0.256
Hb (gr/dL)	12.6 ± 1.7	12.6 ± 1.5	0.972
ACEi/ARB (%)	17.1	16.9	0.987
B Blockers (%)	46.3	50.8	0.658
CCB (%)	53.7	47.5	0.542
Statins (%)	17.1	15.3	0.807

BMI = Body Mass Index, CAD = Coronary Artery Disease, CVD = Cerebrovascular Disease, PAD = Peripheral Artery Disease, BUN = Blood Urea Nitrogen, GFR = Glomerular Filtration Rate, PTH = Parathormone, ACEi/ARB = Angiotensin-converting Enzyme Inhibitors/Angiotensin Receptor Blockers, BB = Beta Blockers, CCB = Calcium Channel Blockers

cIMT values were significantly higher in the fQRS(+) ESRD group than in the fQRS(-) ESRD group. In addition, LVESD, LVEDD, LAD, IVST, PWT diameters, BUN, creatinine and PHT levels were higher in fQRS (+) ESRD patients.

Cardiovascular diseases are still the most common cause of death for ESRD patients [14, 15].The underlying pathophysiological mechanism is atherosclerosis [16]. Therefore, CV risk assessment is very important and vascular imaging is one of the risk assessment methods for atherosclerosis [17]. Carotid artery doppler ultrasound is an inexpensive and non-invasive imaging method. Increased cIMT reflects subclinical atherosclerosis. Various CV guidelines recommend the use of this measurement for the evaluation of target organ damage [18, 19]. Nonetheless, the lack of a standardized cIMT value or consistent measurement methods for increased IMT limits the use of this parameter

Table 2. The cIMT and echocardiographic findings of both study groups

	fQRS (+) Group (n = 41)	fQRS (-) Group (n = 59)	*p* value
EF (%)	59.5 ± 7.7	60 ± 7.2	0.735
LVESD (mm)	33.7 ± 6.6	30.6 ± 5.4	**0.012**
LVEDD (mm)	50.8 ± 6.3	45.9 ± 5.2	**< 0.001**
IVS (mm)	13.2 ± 1.6	10.7 ± 1.6	**< 0.001**
PW (mm)	12.8 ± 1.4	10.5 ± 1.4	**< 0.001**
LAD (mm)	38.2 ± 4.7	34.6 ± 5.4	**0.001**
LVH (%)	94.9	42.4	**< 0.001**
Mean right cIMT (mm)	0.81 ± 0.19	0.62 ± 0.14	**< 0.001**
Mean left cIMT (mm)	0.83 ± 0.19	0.64 ± 0.14	**< 0.001**

EF = ejection fraction, LVESD = left venticle end systolic diameter, LVEDD = left venticle end diastolic diameter, IVS = Interventricular septal diameter, PW = Posterior wall diameter, LAD = left atrial diameter, LVH = left ventricular hypertrophy, cIMT = Carotis intima-media diameter

in clinical trials [20]. Therefore, it may need confirmation with other prognostic markers.

In a study by Benedeto *et al*. [21], cIMT was associated with LV concentric hypertrophy in dialysis patients and was reported to be an independent predictor of CV death, which could be useful for risk classification in the dialysis population. In the current study, a similar relationship between concentric hypertrophy of LV and increased cIMT was found. LVH is a marker of target organ damage, which can be measured non-invasively using echocardiography, and is associated with CV morbidity and mortality in HD patients [22]. In end-organ damage such as LVH, hypertrophy occurs in myocytes due to collagen deposition in interstitial tissue. In this process, myocardial fibrosis may occur in ESRD patients in the presence of LVH and due to progressive increase in renal dysfunction [23].

Myocardial fibrosis occurs as a result of excessive deposition of type 1 collagen fibers into the interstitium, around intramyocardial arteries and arterioles. CKD is one of the reasons for the exaggerated synthesis of collagen by cardiac fibroblasts and myofibroblasts. Exposure to oxidative stress, inflammation and hemodynamic overload starting from the early stages of the CKD and also increased release of cytokines such as cardiotrophin-1 and transforming growth factor-β1 or TGF-β1 contribute to myocardial scar formation. In the more advanced stages of CKD, uremic toxins, anemia, hyperphosphatemia, parathormone excess, and vitamin D deficiency may also cause cardiovascular damage and increased myocardial scarring [24, 25]. Mechanical overload due to LVH and diastolic dysfunction in CKD also leads to exaggerated collagen deposition [26].

fQRS formation reflecting delayed ventricular conduction is a non-invasive parameter used to evaluate atherosclerosis. It is also associated with fibrosis, myocardial scar and ischemia [8]. Several studies have proven the utility of fQRS in predicting major adverse cardiac events (MACE) and all-cause mortality in patients with coronary artery disease [27, 28]. In addition, LV deformation and increased LVH incidence have been reported in hypertensive patients with fQRS. Although there are limited studies on fQRS in patients with ESRD, it has been reported that fQRS may be useful in determining LV dysfunction in renal transplant patients. It has also been shown to be independently associated with normal LVEF and subclinical LV dysfunction in ESRD patients and aortic stiffness has been found to be an important predictor in hemodialysis patients [29, 30]. There is a known association between hypertensive patients with fQRS and impaired diastolic functions [31]. In the current study, increased LVESD, LVEDD, LAD, IVST and PWT parameters in fQRS (+) ESRD patients were associated with impaired diastolic function secondary to LVH and this may lead to myocardial fibrosis. Myocardial scar formation and diastolic dysfunction due to LVH, the presence of fQRS in ECG as well as increased cIMT thickness could demonstrate that ESRD patients may be more prone to atherosclerosis.

Increased BUN, creatinine, and PTH levels reflect impaired kidney function. High values of these parameters in fQRS (+) patients may support the acceleration of the atherosclerotic process.

Limitations

Limitations of this study were primarily the non-randomized, single centered nature and limited number of patients. Due to the lack of a standardized cIMT value, these results may differ from those of large-scale studies. There is a clear need for further more extensive studies of the traditional risk factors and high-risk populations. Unfortunately, due to the non-analytical design of our study we were not able to investigate rigid outcomes in our research. Nevertheless, our study may be helpful during designing hypotheses for advanced trials.

CONCLUSION

In ESRD patients, the presence of fQRS, LVH and increased CIMT are important markers in the evaluation of the inflammatory process of atherosclerosis. Increased cIMT is related with fQRS presence in ESRD patients.

Authors' Contribution

Study Conception: TD; Study Design: MY; Supervision: TD; Funding: TD; Materials: İD; Data Collection and/or Processing: TD; Statistical Analysis and/or Data Interpretation: OÇ; Literature Review: MK; Manuscript Preparation: LB and Critical Review: YK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Increase in scabies incidence: a retrospective cohort study

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ABSTRACT

Objectives: Recently, there are studies from many countries reporting that scabies incidence has increased. Similarly, there was an increase in the frequency of scabies admitted to our outpatient clinic. Our aim in this study is to draw attention to the increasing incidence of scabies in our region.

Methods: The cases diagnosed with scabies in dermatology outpatient clinic between 2010-2019, their demographics and treatments were retrospectively analyzed.

Results: It was detected that 949 (0.55%) scabies cases were diagnosed between 2010-2019. When it was analyzed by years, the 3.5 times increase in cases with scabies in 2014 compared to the previous year was found statistically significant ($p < 0.05$). When data from 2018 was compared with the data from 2010, an increase in the number of cases by 7.6 times was detected in the outpatient numbers with scabies and by 4.7/1000 person-years times in the general population ($p < 0.05$). Besides, it was determined that the Syrian asylum seekers started to apply to our outpatient clinic as of 2014, and that the scabies incidence was very high within the same year in these patients (27.6%). It was detected that permethrin was prescribed by 88.4% to 868 cases whose treatment details were achieved, and that the ratio of response to treatment was 95.8%.

Conclusions: The results we obtained reveal that there was an increased scabies incidence from 2014 in our region. The necessity to immediately and effectively intervene the affected cases to prevent possible epidemic attacks has formed.

Keywords: scabies, dermatology, incidence, scabicide resistance, Syrian asylum seekers

Scabies is a common pruritic skin infestation caused by the Sarcoptes scabiei mite [1]. The most common symptom is rash which increases at night [2]. According to Vos *et al*. [3], more than 200 million people around the world suffer from this ectoparasitic infection. The disease burden affects 0.2% to 71.4% of the general population depending on the region [4]. Scabies is a global issue and it is an important cause of morbidity due to its high infectiousness [1]. The infection can spread from person to person via direct skin contact [5]. There are many factors that affect the spread of scabies, including overcrowding, hygiene, age, socioeconomic status, and season [5]. It is critical to know the epidemiological data on this parasite which may easily lead to epidemics, and to take the required measures [6]. In the recent years, there are studies from many countries reporting that scabies incidence has increased [3]. Similarly, there was an increase in the frequency of scabies admitted to our outpatient clinic. We hypothesized that there is an increase in scabies cases in our region. In this study, it is intended to determine the status of scabies in our region by reviewing the outpatient clinic records.

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METHODS

This study is a hospital-base and retrospective cohort study. The study was approved by the Derince Research and Training Hospital Non-invasive Research Ethics Committee (2019-64 / 8.8.2019). All these procedures were performed in accordance with the principles of the Declaration of Helsinki. The files of the patients who were examined at Dermatology Outpatient Clinic of Darıca Farabi Training and Research Hospital between January 1, 2010 and January 1, 2019, and who were diagnosed with scabies were reviewed retrospectively.

The control examinations and multiple applications of the same patient for scabies diagnosis were considered as one. The suspected cases were excluded from the study.

Statistical Analysis

Statistical analyses were performed with SPSS 22 (SPSS Inc., Chicago, Illinois, USA) program. Descriptive statistics were presented by frequencies, percentages, mean ± standard deviation (min-max) values. The normal distribution of the variables was tested by Kolmogorov smirnov. Comparisons between groups of independent samples were assessed with independent-samples T test, ANOVA and Z test. Categorical data were compared by Pearson's chi-square test. The results were evaluated in a confidence interval of 95% and a significance level of $p < 0.05$.

RESULTS

Nine hundred forty-nine (0.55%) of 173.136 cases confirmed by dermatoscopy or light microscopy between 2010-2019 were diagnosed with scabies. The mean age of the patients was 29.78 ± 19.2 (0-84) years. Among the cases, 546 (57.5%) were female, 402 (42.4%) were male and the mean age was 31.48

Table 1. Gender distribution based on age groups

Age	Patient		Female		Male	
	n	%	n	%	n	%
0-18	341	35.9	175	18.4	165	17.4
19-40	334	35.2	182	19.2	152	16
41-65	234	24.7	167	17.6	67	7.1
> 65	40	4.2	21	2.2	18	1.9

Table 2. Data regarding the cases diagnosed with scabies based on population and number of examination

Years	Cases diagnosed with scabies	District population	1,000 Person-years	Total number of patients	%
2010	34	146.896	0.23	24903	0.14
2011	24	152.542	0.16	30167	0.08
2012	22	157.304	0.14	11272	0.2
2013	42	164.385	0.26	15806	0.26
2014	163	173.139	0.94	17859	0.91
2015	146	182.710	0.8	17005	0.86
2016	127	191.123	0.66	15983	0.8
2017	172	198.153	0.87	19634	0.88
2018	219	201.468	1.09	20507	1.07
Total	949	1.567.720	0.61	173136	0.55

± 19.2 (0-84) years, 26.05 ± 18.75 (0-82) years, respectively. There was a statistically significant difference between the patient groups in terms of gender and mean age ($p < 0.05$). The mean age of patients with scabies was found to decrease from 33.35 years in 2010 to 26.67 years in 2018 ($p < 0.05$).

One hundred seven (12.3%) scabies cases were detected among 1.192 Syrian asylum seekers examined between 2014-2019, and the mean age of the cases was 18.8 ± 13.01 years. When the mean age of all cases and the asylum seekers is compared, the difference was found statistically significant ($p < 0.05$). 78 (53.1%) of the Syrian cases were female, 69 (46.9%) were male and the mean age was 20.47 ± 14.3 years, 17 ± 11.2 years. All patients diagnosed with scabies were analyzed in four groups which are 0-18 years, 19-40 years, 41-65 years and above 65 years (Table 1). 341 (35.9%) of the cases were children and the mean age of them was 9.8 ± 5.3 years.

The population of the district, total number of cases, number of cases with scabies, number of cases per 1000 people and the incidence were calculated separately by year (Table 2, Fig. 1). It was detected that the number of cases diagnosed with scabies increased by 3.5 times in 2014 compared to 2013, and this increase was found statistically significant ($p < 0.05$). When data from 2018 was compared with the data from 2010, an increase in the number of cases by 7.6 times was detected in the outpatient numbers and by 4.7 times per 1000 people ($p < 0.05$). The incidence of scabies among Syrian asylum seekers in 2014 was 27.6% and its effect on the overall percentage was 0.13% (Table 3). The prevalence of scabies in asylum seekers between 2014-2018 was 12.33%, and its effect on the overall percentage was 0.08%. It was found that scabies decreased in the asylum seekers over the years

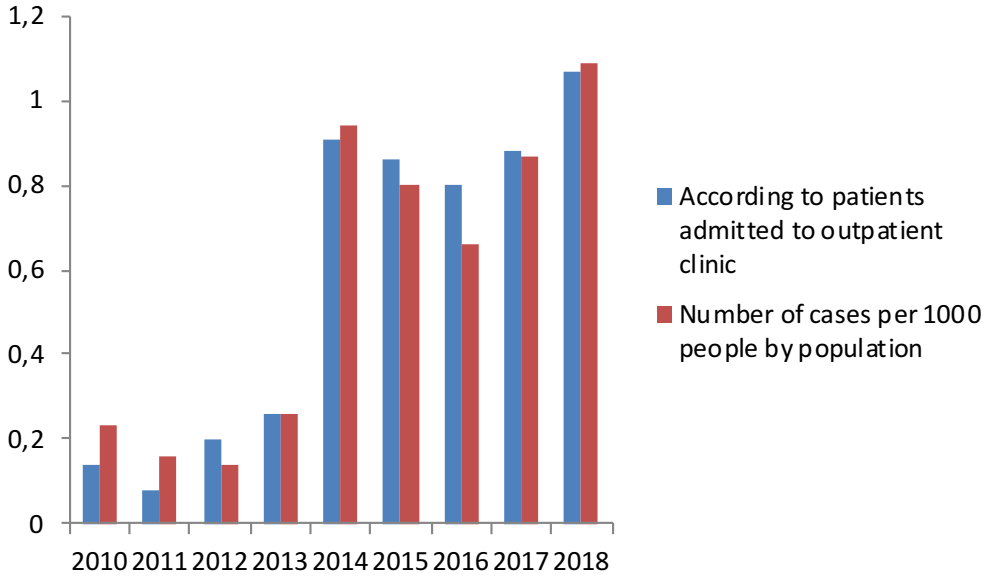

Fig. 1. **Scabies incidence by years.**

Table 3. **Syrian patients diagnosed with scabies by years**

Years	Total Syrian cases (n)	Syrian cases diagnosed with scabies		
		n	%	Effect on overall %
2014	87	24	27.6	0.13
2015	112	31	27.7	0.18
2016	192	26	13.5	0.17
2017	367	44	12	0.22
2018	434	22	5.1	0.09
Total	1192	147	12.33	0.08

Table 4. Ratio of permethrin preference and non-response

Years	Ratio of permethrin preference, %	Ratio of non-response to treatment, %
2013	95.1	2.6
2014	95.7	2.6
2015	86.3	5.5
2016	92.1	4.3
2017	84.3	4.1
2018	84	4.4
Total	88.4	4.0

and that this decrease was statistically significant ($p < 0.05$).

From the database, the treatment details of 868 cases in total as of 2013 were achieved. According to this, 767 cases (88.4%) were prescribed topical permethrin (5% cream or lotion) in the first examination, 88 cases (10.1%) were prescribed benzyl benzoate (20% cream), 13 cases (1.5%) were prescribed topical sulfur (10% ointment). It was determined that 25 cases who were initiated permethrin were prescribed benzyl benzoate and 6 cases were prescribed sulfur due to insufficient treatment. It was determined that 13 cases, who started to take benzyl benzoate during the first treatment without response, were prescribed permethrin and 1 case was prescribed sulfur. According to this, treatment failure for permethrin was 4.0% and 15.9% for benzyl benzoate. There was no statistically significant difference in response rates to treatment by years ($p > 0.05$). Topical permethrin treatment preferences and response rates by year are given in Table 4.

DISCUSSION

In this study, cases diagnosed with scabies in dermatology outpatient clinic between 2010 and 2019 were retrospectively scanned. The mean age of 949 (0.55%) cases with this diagnosis was 29.78 ± 19.2 years. Likewise, in the study of Anderson and Strowd [1], the mean age of the cases with scabies was detected as 27 years. In addition, we also found that the mean age of patients with scabies has decreased significantly over the years, similar to the study of Aktaş *et al* [7]. Although scabies occurs in both genders, while some researches indicate that it is dominant among males, others show that it is more common among females [8]. In our study, it was detected that 57.5% of the cases were female and 42.4% were male, and their mean age was 31.48 ± 19.2 years, 26.05 ± 18.75 years, respectively. The number of female patients were significantly higher than male patients, and the difference in terms of mean age was statistically significant. A global meta-analysis is recommended in order to understand the role of gender in the global prevalence of scabies better.

The current literature indicates that scabies is more prevalent in the pediatric age group (between 0 to 18 years) than adults [1]. Similarly, in our study the ratio of patients under the age of 18 was higher (35.9%) compared to other age groups.

When the epidemiological data related to scabies in our country are reviewed, while the prevalence of the disease reported in studies between 1970-2000 varies between 1.74%-11.5%, it is seen to be below 1% in studies conducted after 2000 [9-12] The number of current studies conducted in our country on the epidemiology of scabies is very few. In the retrospective study covering the period between 2006-2017 conducted by Çetinkaya *et al*. [6] in Kayseri the number of cases per population was at the highest level in 2014. In the retrospective cohort study covering the period 2013-2017 which was conducted by Aktaş *et al*. [7] in Karabük, the scabies was determined to increase progressively. In our study, it was determined that the number of cases with scabies between 2010-2013 was below 0.26% and it increased by 3.5 times in 2014 compared to the previous year and exceeded 0.9%, and this progress continued in the following years.

Recently, many studies from numerous countries reported an increase in the scabies incidence. Sunderkötter *et al*. [13] reviewed the reports regarding the increased incidence of scabies in Germany. According to this, it was reported that the number of patients treated for scabies increased by 200% from 2014 to 2016 in Nordrhein region. According to the research by a medical company, the benzyl benzoate and permethrin prescriptions increased by 60% in 2017 compared to 2016. It was also indicated that the sales of permethrin, allethrin, benzyl benzoate and invermectin group by German pharmacies increased by four times between 2012-2017 [13]. In Nigeria, while the scabies

incidence in 2012 was 0.85%, it was reported as 6.67% in 2017 [14]. In Norway, it was reported that the sales of drugs and consultations for scabies increased by three times between 2006-2018 [15].

The increase in scabies incidence was associated with the occurrence of the factors known to support the scabicide resistance, increase in risk groups (children, immunosuppressed and inactive elders, risk groups for sexually transmitted diseases, immigrants and refugees) or increase in prevalence [13]. There are no results to prove this in our age-specific data. A considerable number of Syrian asylum seekers are living in Darıca and neighboring districts. It was determined that the asylum seekers started to apply to our hospital as of the beginning of 2014, and that the scabies was very prevalent among the patients during that year (27.6%). The change in economical and socio-cultural background in cases of war and immigration leads to many health problems, especially the infectious diseases [16]. The infectious diseases which previously occurred in certain regions may be transferred to the immigrated area through immigration, and this may lead to the increase of the incidence of the disease [17]. It was reported that 7 thousand 600 scabies cases were recorded in Syria which is affected by the war between 2012-2015, and that 50% of the population in Aleppo were affected by the scabies epidemic [18]. Within the context of refugee crisis, the previous observational studies indicate that scabies is one of the most common diseases and the extent to which it may spread [19]. Rapid spread of scabies has been described in Lebanon with regards to migrant populations and asylum seekers, some of which include the time frame of the current Syrian crisis [20]. In a study conducted in Germany, scabies were detected in 16 of 52 patients, 40% of whom were Syrian asylum seekers [21]. In a similar study conducted in Brussels, in 3907 cases of which 20% consisted of Syrian asylum seekers, the skin symptoms were detected by 9% and the majority of the cases were reported to be scabies [22]. In a study conducted in Lebanon in 2013, it was reported that infectious skin diseases including scabies were detected in 47% of 90 thousand displaced Syrian patients [23]. Although the incidence of scabies among the asylum seekers significantly decreased during the 5-year period when they lived in our region, 2018 data (5.1%) show that this is very high for today's conditions and that they are still under threat in terms of scabies.

In the treatment of scabies, oral ivermectin, topical permethrin and benzyl benzoate are the most frequently used agents [24]. In our country, oral ivermectin is not licensed. Permethrin is considered as the most preferred treatment in many countries today due to its safety and low toxicity, and the cure ratios vary between 90% and 98% [24-27] In our study, it was detected that the efficiency of permethrin was 96.0%. The treatment efficiency for benzyl benzoate was reported as 80% [24]. We detected this rate as 84.1%. The results we obtained in terms of treatment efficiency comply with the previous clinical studies. Although an in vitro prospective study indicated that mites have increasing tolerance against permethrin, there are no reports validating the in vivo resistance [13,28,29] Based on our six-year successful data, we consider that there is no permethrin resistance and it should be the first choice in scabies treatment. Even though we achieved perfect cure results, we believe that our data should be supported with prospective studies to determine whether the increasing number of cases in our region is associated with the scabicide resistance.

Limitations

The most important restrictions of our study are its retrospective and single-centered design. Besides, the data is limited to those recorded in the automation system. Failure to reach the treatment data of the cases before applying to a dermatologist, insufficient disease severity data and background information of the cases prevent us from achieving more detailed and objective epidemiological data.

CONCLUSION

The results we obtained reveal that there is an increased scabies incidence in our region. Although this increase occurred concurrently with the immigration movements, we do not have clear data to explain the actual cause. Based on our six-year data, permethrin is still reliable and efficient in the treatment of scabies. Nevertheless, we believe that our data should be supported with prospective studies to determine the contribution of scabicide resistance to the number of increased cases.

According to the literature, scabies maintains its global increase and continues to be a public health issue. A necessity to implement an effective central notification system for scabies disease for which notification is not mandatory in our country has formed. A multidisciplinary approach is essential to prevent possible epidemic attacks. Implementing an effective surveillance system may lead to decrease in healthcare cost in addition to improved productivity.

Authors' Contribution

Study Conception: FB; Study Design: FB; Supervision: FB; Funding: FB; Materials: FB; Data Collection and/or Processing: FB; Statistical Analysis and/or Data Interpretation: FB; Literature Review: FB; Manuscript Preparation: FB and Critical Review: FB.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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The European Research Journal 2021;7(5):495-500 Original Article

DOI: 10.18621/eurj.802982 *Nephrology*

The incidence of hypophosphatemia in the early posttransplant period in renal transplant recipients and its association with graft function

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ABSTRACT

Objectives: To investigate the prevalence of posttransplant hypophosphatemia in the early posttransplant period among renal transplant recipients in relation to its impact on renal graft function.

Methods: A total of 78 renal transplant recipients who were transplanted between January 2016 and March 2020 were included in this retrospective single center study. Data on laboratory findings (phosphate, creatinine, estimated glomerular filtration rate [eGFR], albumin, serum corrected calcium and parathyroid hormone [PTH] levels) at pre- and posttransplant 3 month follow up period were recorded.

Results: Hypophosphatemia was detected in 16 (20.8%), 13 (16.7%) and 7 (9.1%) patients at the posttransplant day 10, month 1 and month 3, respectively. Posttransplant day 10 and day 30 measurements revealed significantly lower serum creatinine values ($p < 0.001$ and $p < 0.07$, respectively) and significantly higher eGFR values ($p = 0.009$ and $p = 0.036$, respectively) in the hypophosphatemic group compared to the normophosphatemic group. Serum phosphate displayed linear relationship with creatinine at day 10 ($r = 0.687$, $p < 0.001$), day 30 ($r = 0.301$, $p = 0.007$), while not correlated with PTH levels at posttransplant day 10, day 30 and day 90.

Conclusions: Our findings suggest that hypophosphatemia is common in the early posttransplant period, particularly first month after kidney transplantation, being associated with better renal graft function.

Keywords: Hypophosphatemia, kidney transplantation, graft function

Hypophosphatemia is a prevalent complication observed in 40-90% of renal transplant patients in the posttransplant first month [1]. In chronic kidney disease (CKD), along with a decline in eGFR, phosphaturic hormones such as parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF-23) increase in response to phosphate retention and inhibit renal tubular phosphate reabsorption [2, 3]. As kidney function recovers after kidney transplantation, the accumulated FGF-23 and PTH exaggerate renal tubular phosphate leak, leading to phosphate depletion. With normalization of PTH and FGF-23 levels toward baseline, serum phosphate levels gradually increase and reach the normal limits within 12 months [4-6].

Although hypophosphatemia is considered likely to be associated with a good graft function and prolonged graft survival, the clinical relevance of posttransplant hypophosphatemia remains unclear in terms of graft survival exact [7].

This study aimed to evaluate the relationship be-

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tween serum phosphate levels and graft function in renal transplant patients within the first 3 months of posttransplant period.

METHODS

Study Population

Seventy eight consecutive adult patients who underwent renal transplantation between January 2016 and March 2020 were included in this retrospective single center study. Inclusion criteria were receiving ABO-compatible first-time kidney transplantation at least 1 year ago, while exclusion criteria were being under the age of 18 and history of pretransplant parathyroidectomy. There was no current or previous noncalcium-containing phosphate binder, vitamin D analog, or calcimimetic use in any of the patients. Written informed consent was obtained from each subject following a detailed explanation of the objectives and protocol of the study which was conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" and approved by the Ufuk University Faculty of Medicine Ethics Committee (Protocol no: 20200703/2).

Assessments

Data on patient demographics, clinical and laboratory findings, tacrolimus levels and follow-up records were collected from the hospital database. Patients were followed up during the first 90 days of posttransplant period and data on routine laboratory tests (creatinine, phosphate, calcium, albumin, parathyroid hormone levels) were recorded before transplantation and on posttransplant 10th day, 1st month and 3rd months. Posttransplant hypophosphatemia was defined as serum phosphate level < 2.3 mg/dL. Serum calcium was corrected based on the following equation: Corrected Ca (mg/dL) = Serum Calcium + [(4.0 – albumin (g/dL)) × 0.8)] [8]. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula [9].

Treatments

When indicated anti-thymocyte globulin (ATG, Grafalon Neovii) was administered at 100 mg/g dose for 3 days. Following total 1500 mg intravenous methyl-prednisolone, all patients were administered prednisolone (0.8 mg/kg/day, orally). Prednisolone dose was tapered to 30 mg/day at 1 month, 20 mg/day at 2 months and 5 mg/day after 3 months. In the maintenance treatment phase, calcineurin inhibitor [tacrolimus (Tac); 0.1 mg/kg/day, 2 doses per day] and antiproliferative agent (mycophenolate mofetil; maximum 2 g/day or mycophenolate sodium; maximum 1440 mg/g) were used along with prednisolone. Tac doses were titrated as needed to achieve target blood levels. In case of acute rejection, renal biopsy was performed and treatment (pulse methyl-prednisolone, ATG, plasmapheresis, and intravenous immunoglobulin treatments alone or in combination) was administered according to Banff criteria [10].

Statistical Analysis

Statistical analysis was made using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY). The Student-t test was used to compare the findings in hypophosphatemic and normophosphatemic groups for each time period. The Pearson correlation analysis was used to measure the strength of a linear association between serum phosphate and other variables and then Simple Linear Regression analysis was performed for further analysis. Data were expressed as mean± standard deviation (SD), median (interquartile range) and percent (%) where appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

Of the patients, 21.8% were female and 78.2% were male. The mean age was 43 ± 14 years and median follow-up period was 28 (ranged, 15 to 40) months. The underlying causes of end-stage renal disease involved chronic glomerulonephritis in 32 (41%) patients, hypertension in 17 (20.8%) patients, type 2 diabetes mellitus in 12 (15.4%) patients and secondary amyloidosis in 10 (12.8%) patients, while nephrolithiasis, polycystic kidney disease and no detectable cause were noted in 2 (2.6%), 2 (2.6%) and 3 (3.8%) patients, respectively. During follow-up, acute allograft rejection occurred in 16 (20.5%) patients. Dialysis type, number of tissue adaptation, type of transplantation, type of induction and maintenance immunosuppression treatment features are shown in Table 1.

The mean pretransplant phosphate level was 4.2 ±

Table 1. Patient demographics and baseline characteristics

Variables	n = 78
Gender, F/M, n (%)	17/61 (21.8/78.2)
Age, (year, mean ± SD)	43 ± 14
Type of dialysis, n (%)	
Preemptive	40 (51.3)
Hemodialysis	37 (47.4)
Peritoneal dialysis	1 (1.3)
Transplant type (live/cadaver)	75/3 (96.2/3.8)
Miss-Match count, n (%)	
0 MM	3 (3.8)
1 MM	7 (9.0)
2 MM	10 (12.8)
3 MM	26 (33.3)
4 MM	14 (17.9)
5 MM	9 (11.5)
6 MM	9 (11.5)
Transplant duration (month, median, IQR)	28 (15-40)
Induction treatment, n (%)	
ATG	34 (43.6)
None	44 (56.4)
Maintenance treatment	
Tac+MMF	61 (78.2)
Tac+MFA	17 (20.8)
Acute rejection, n (%)	16 (20.5)

SD = standard deviation, F =female, M = male, MM = miss-match, Tac = tacrolimus, ATG = anti-thymocyte globulin, MFA = mycophenolic acid, MMF = mycophenolate mofetil, IQR = interqartile

1.37mg/dL, which decreased to 3.2 ± 1.01mg/dL, 3.2 ± 0.86 mg/dL, and 3.4 ± 0.70 mg/dL at the posttransplant day 10, month 1 and month 3, respectively. Hypophosphatemia was detected in 16 (20.8%), 13 (16.7%) and 7 (9.1%) patients at the posttransplant day 10, month 1 and month 3, respectively. Mean posttransplant levels of creatinine significantly decreased to 1.65 ± 1.10 mg/dL, 1.37 ± 0.60 mg/dL, and 1.39 ± 0.43mg/dL at the posttransplant day 10, month 1 and month 3, respectively. The mean pretransplant PTH level was 418.14 ± 400.75 ng/L and decreased to 184.55 ± 135.38 ng/L, 202.46 ± 132.66 ng/L, and 134.31 ± 95.62 ng/L at the posttransplant day 10, month 1 and month 3, respectively. When compared to pre-transplant laboratory parameters, significant difference was noted in post-transplant parameters for 10th day, 1st month and 3rd month ($p < 0.05$) (Table 2).

Posttransplant day 10 and day 30 measurements revealed significantly lower serum creatinine values ($p < 0.001$ and $p < 0.07$, respectively) and significantly higher eGFR values ($p = 0.009$ and $p = 0.036$, respectively) in the hypophosphatemic group compared to the normophosphatemic group (Table 3, Fig. 1 and Fig. 2).

Serum phosphate displayed linear relationship with creatinine at day 10 (r = 0.687, $p < 0.001$), day 30 (r = 0.301, $p = 0.007$) and day 90 (r = 0.070, $p = 0.548$) and inverse relationships with eGFR at day 10 (r = -0.461, $p < 0.001$), day 30 (r = -0.157, $p = 0.171$)

Table 2. Laboratory parameters before and after transplantation

	Pretransplant	Posttransplant		
Variables, mean ± SD	Day -1	Day +10	Day +30	Day +90
P (mg/dL)	4.2 ± 1.37	3.2 ± 1.01	3.2 ± 0.86	3.4 ± 0.70
Cr (mg/dL)	4.62 ± 1.62	1.65 ± 1.10	1.37 ± 0.60	1.39 ± 0.43
eGFR (ml/min/1.73m^2)	15.39 ± 5.69	60.26 ± 25.59	67.33 ± 22.24	67.24 ± 23.83
c-Ca (mg/dL)	9.89 ± 0.64	9.63 ± 0.89	9.73 ± 0.43	9.53 ± 0.89
PTH (ng/L)	418.14 ± 400.75	184.55 ± 135.38	202.46 ± 132.66	134.31 ± 95.62

Data are shown are mean±standard deviation. c-Ca = corrected calcium, Cr = creatinine; eGFR = estimated glomerular filtration rate, P = phosphate; PTH = parathyroid hormone

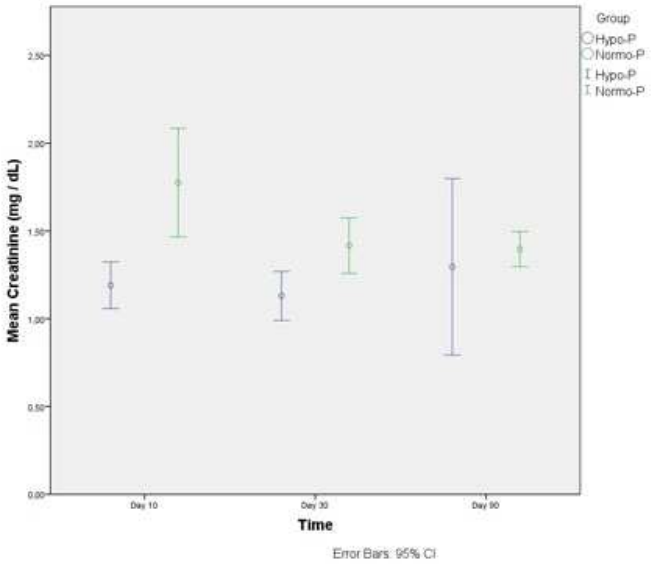

Fig. 1. Comparison of posttransplant creatinine values of hypophosphatemic and normophosphatemic groups.

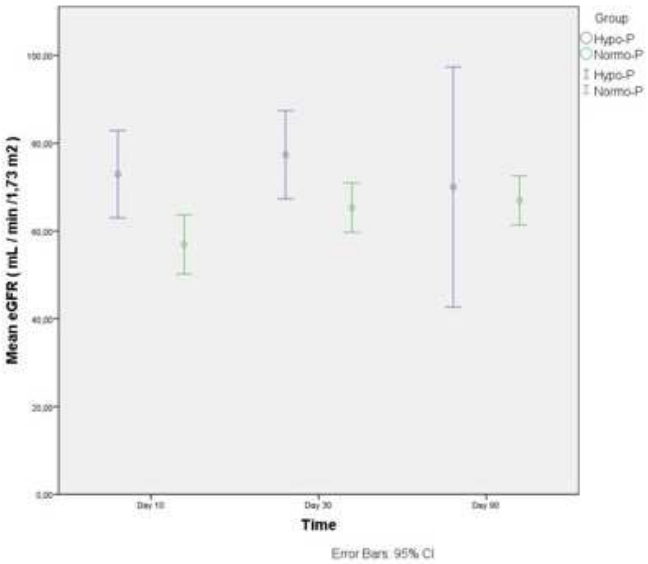

Fig. 2. Comparison of posttransplant eGFR values of hypophosphatemic and normophosphatemic groups.

and day 90 (r = -0.013, p = 0.911). No significant correlation was noted between serum phosphate and PTH levels at day 10 (r = -0.134, p = 0.416), day 30 (r = -0.262, p = 0.163) and day 90 (r = -0.184, p = 0.388).

DISCUSSION

In the early posttransplant stage, the relatively high FGF-23 and PTH concentrations in relation to restored renal excretory capacity of phosphate may result in hypophosphatemia, especially within the first year [11]. In a past study by Ghorbani *et al*. [5] in 50 kidney transplant patients, hypophosphatemia was reported in 42% of patients in the first posttransplant month. In the current study, hypophosphatemia was noted in 13 (16.7%) patients in the in the first posttransplant month.

Our findings related to association of the development of hypophosphatemia with a lower creatinine and higher eGFR values seems to be in accordance with consideration of good graft function to be accompanied by an increased renal capacity to excrete phosphate in early after transplantation. Identification of lower creatinine levels and higher eGFR values in our hypophosphatemic patients than in normophosphatemic patients at posttransplant day 30 indicates an inverse correlation between serum phosphate levels and kidney function. Likewise, in a past study by Seifi *et al*. [12] with 237 transplant patients, hypophosphatemia was reported in 58% of patients within the first 2 months of the posttransplant period, while au-

thors also noted significantly lower serum creatinine levels in hypophosphatemic vs. normophosphatemic patients [12]. In a past study among 90 renal transplant patients, presence of hypophosphatemia at the post-transplant first month and 3rd month but not at the posttransplant 12th month was reported to be an independent predictor of good kidney survival [3].

Elevated PTH levels before transplantation have been shown to decline during the first 3 months after kidney transplantation. High PTH levels can be observed in 30-60% of kidney transplant recipients with good allograft function in the 1st year after kidney transplantation. PTH production cannot reduce instantly after kidney transplantation, and along with the increase in eGFR, the sudden change in the definition of hyperparathyroidism may complicate the interpretation [13]. Similar to our findings, there studies in the literature indicated no significant difference between normo- and hypophosphatemic patients in terms of PTH levels [5, 14]. Bhan *et al*. [15] reported that pre-transplant PTH elevation was not a risk factor for post-transplant hypophosphatemia, while FGF-23 levels were independently associated with decreased serum phosphate levels. In the current study, since FGF-23 levels were not measured, the association between FGF-23 levels and posttransplant hypophosphatemia could not be analyzed.

The high-dose steroids and tacrolimus are considered to be associated with renal phosphate wasting. However, while hypophosphatemia is commonly noted after kidney transplantations, it does not typically develop after other solid organ transplants despite use of similar and often higher doses of immunosuppressive regimens [16, 17]. In the current study, while almost all patients were on an identical immunosuppressive regimen, only some patients developed hypophosphatemia. Accordingly, immunosuppressive agents, whilst may induce phosphaturia after renal transplantation, seem unlikely to be the primary cause of hypophosphatemia.

Limitations

The major limitations of the current study seem to be the small sample size and lack of data on FGF-23 levels, 25(OH)D vitamin levels and fractional phosphate excretion at pre- and post-transplant period as well as on the dietary phosphate intake.

Table 3. Association of hypophosphatemia with creatinine, eGFR and PTH levels at the posttransplant 10, 30 and 90 days

	Day 10			Day 30			Day 90		
Variables	**Hypo-P**	**Normo-P**	***p value***	**Hypo-P**	**Normo-P**	***p value***	**Hypo-P**	**Normo-P**	***p value***
P	1.98 ± 0.27	3.51 ± 0.88	**< 0.001**	2.02 ± 0.25	3.48 ± 0.71	**< 0.001**	2.03 ± 0.28	3.49 ± 0.58	**< 0.001**
Cr	1.19 ± 0.25	1.78 ± 1.21	**< 0.001**	1.13 ± 0.23	1.42 ± 0.64	**0.007**	1.30 ± 0.54	1.40 ± 0.42	0.556
eGFR	72.93 ± 18.66	56.94 ± 26.24	**0.009**	77.38 ± 16.62	65.33 ± 22.77	**0.036**	70.00 ± 29.59	66.96 ± 23.42	0.750
PTH	226.56 ± 180.65	175.37 ± 125.16	0.498	170.10 ± 128.47	210.55 ± 135.13	0.514	186.70 ± 100.16	132.03 ± 97.10	0.587

Data are shown are mean±standard deviation. Cr = creatinine; eGFR = estimated glomerular filtration rate; P = phosphate; PTH = parathyroid hormone; SD = standard deviation

CONCLUSION

In conclusion, hypophosphatemia is frequently seen after renal transplantation in relation to the functional performance of the transplanted kidney. Our data also suggest that the impact of hypophosphatemia on graft survival is influenced by the time after kidney transplantation. Further prospective, larger scale, controlled and multicenter cohort studies are needed to investigate the prevalence of hypophosphatemia in the early posttransplant period and its correlation with graft function.

Authors' Contribution

Study Conception: EIS; Study Design: EIS; Supervision: EIS; Funding: EIS; Materials: EIS; Data Collection and/or Processing: EIS; Statistical Analysis and/or Data Interpretation: EIS; Literature Review: EIS; Manuscript Preparation: EIS and Critical Review: EIS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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Retrospective investigation of postoperative mid-term results of cryoablation and radiofrequency ablation methods used in atrial fibrillation surgery treatment

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ABSTRACT

Objectives: Atrial fibrillation (AF) is the most common arrhythmia encountered and is usually seen in patients scheduled for coronary artery bypass and mitral valve surgery. Radiofrequency ablation and cryoablation are two methods used in AF surgery and proven efficacy. In this study, cryoablation and radiofrequency ablation methods were compared in terms of clinical outcomes, efficacy and safety.

Methods: Between November 2011 and September 2017; 99 patients with AF who underwent radiofrequency ablation or cryoablation during open heart surgery were included in this study with 2 groups. The patients who underwent cryoablation were defined as Group I (n = 40), and the patients who underwent radiofrequency ablation as Group II (n = 59). Preoperative, perioperative, early and mid (1 year) postoperative period characteristics of the groups were analyzed.

Results: The mean age was 60.6 ± 9 years in Group I and 60.7 ± 9.1 years in Group II ($p = 0.960$). When the operative values were examined, ablation time and cross-clamp time was found to be low in Group II and a statistically significant difference was found ($p < 0.001$ and $p = 0.043$; respectively). When the rhythms of the postoperative first year controls are examined, sinus rhythm was observed in 34 (85%) patients in Group I and 50 (84.7%) patients in Group II. There was no statistically significant difference in return to sinus rhythm in the first year ($p = 0.975$).

Conclusions: The success rates of these two methods used in AF surgery are effective but they are not superior to each other.

Keywords: Atrial fibrillation, cryoablation, radiofrequency ablation, surgical ablation

Atrial fibrillation (AF) is a common arrhythmia in the society. It was first described in 1909 by Thomas Lewis. While the incidence in the general population is between 0.4% and 2%, this rate reaches 10% over the age of 60. This rate was reported to be between 30% and 84% in patients scheduled for surgery for mitral valve, and 5% in patients scheduled for surgery for coronary heart disease [1].

AF has been perceived as a benign arrhythmia for many years; therefore, treatment options have been

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limited. However, several previous studies showed that AF increases the probability of stroke by six times, doubling the mortality due to cardiovascular causes. Even in the absence of stroke, it was shown that AF probably causes cognitive impairment as a result of silent lacuner and cortical infarctions or hypoperfusion. Persistent AF was associated with doubled mortality due to all causes and cardiovascular disease-related [2-4].

Approaches in surgical treatment targeted to stop uncontrolled electrical triggers in the atrium and to ensure atrial contraction function. For this purpose, left atrial isolation procedure, catheter ablation of sense node, corridor procedure, pulmonary button isolation, atrial compartment operations were applied, respectively throughout the history. Finally, the surgery that was called "Maze III Procedure", which was developed by Cox and which was modified twice, became the gold standard for AF treatment with 99% success. Today, radiofrequency (RF) ablation and cryoablation methods are used widely in the surgical treatment of AF [5]. Success of AF treatment with isolated left atrial RF ablation and cryoablation has been reported to be 70–85% [6-8].

The purpose of this study was to determine the efficacy and reliability, and early and mid-term clinical results after RF ablation and cryoablation methods used for AF therapy in patients who have AF rhythm in the preoperative period and have undergone open heart surgery.

METHODS

A total of 99 patients at the University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa, Turkey, between November 2011 and September 2017, who underwent open heart surgery with cardiopulmonary bypass (CPB), who had AF in the preoperative period, and who underwent RF ablation or cryoablation during the surgery were included in the study in 2 groups (Group I: Cryoablation, Group II: RF ablation). The study was designed retrospectively based on the database, and was approved with the decision of Uludag University Faculty of Medicine Clinical Research Ethics Committee on 21.11.2017 with the number 2017-17/3.

The preoperative, perioperative, early and mid (1 year) postoperative period features of patients were recorded. Patients with endocarditis, sick sinus syndrome, calcification in the left atrium wall, who did not admit to our hospital for routine outpatient follow-ups after discharge, and who did not complete their first 1-year follow-ups were excluded from the study. All patients were examined with transthoracic echocardiography (TTE) in the preoperative period. In the echocardiography unit of our hospital, left atrium (LA) diameter and ejection fraction (EF) were calculated using the Teichholz formula from two-dimensional parasternal long axis images with the G.E Electronics Vivid 7 Echocardiography device. The pulmonary artery pressure (PAP) was measured with continuous wave (CW) method through the tricuspid valve in patients who had tricuspid insufficiency. In patients who did not have tricuspid insufficiency, it was measured with pulmonary flow acceleration time.

Surgical Technique

Aorta-bicaval cannulation was performed after median sternotomy, and CPB was started after the ACT (activated clotting time) was > 400. After cross-clamping, if there was coronary bypass grafting (CABG) in the surgical procedure to be performed, distal anastomosis of saphena grafts was performed firstly. If the surgery to be performed was valve surgery, left atriotomy was performed.

After left atriotomy, firstly, left atrium and appendix were evaluated. The first thing to do was thrombectomy in cases with thrombus in the left atrial appendix, and the left atrial appendix was ligated internally with 4/0 prolene suture. If valve replacement was planned, the mitral valve was excised (if possible, posterior leaflet was preserved). The right pulmonary veins were circled starting from the incision made from the interatrial groove, then the left pulmonary veins were circled in one single circle to form a set of lesions. Then another set of lesions was created to combine the two candidates that included the right and left pulmonary veins from the superior segment. This line was positioned towards the left atrium ceiling as much as possible to avoid possible esophageal damage. Another ablation line was created to combine the left pulmonary veins and the mitral valve posterior annulus after the ablation line extending from the left atrial appendix to the left superior pulmonary vein was created. Another line that started exactly in the middle

of this line was extended towards the atrium base to prevent "re-entry" waves that might occur between atriums through the coronary sinus.

As the precaution for this, ablation was performed before additional surgical procedure in order not to damage the suture lines. In addition, wet gauze was placed in the LA posterior before the ablation to prevent possible esophageal damage, and the transesophageal echocardiography (TEE) probe was withdrawn (if any). The ablation line was directed towards P2-P3 to avoid damage to the circumflex artery during the ablation process. The Cardioblate CryoFlex Surgical Ablation Probe (Medtronic Inc., Minneapolis, MN, USA) was used in Group I, and the Irrigated Unipolar Cardioblate® Surgical Ablation Pen was used in Group II (Medtronic Inc., Minneapolis, MN, USA).

The concomitant surgery procedure was performed after the ablation procedure was completed. The cross-clamp was removed after the surgical procedure was completed, and CPB was terminated. Temporary epicardial "pacemaker" wires were placed in patients at the end of the surgery against AV block risk.

Postoperative Medical Treatment and Follow-up

In our clinic, our medical treatment protocol for patients who undergo surgical ablation is administering intravenous Amiodaron (1200 mg) in the first 24 hours postoperatively, to every patient without discrimination. Half of this treatment dose (600 mg) is given in the form of loading dose after the cross-clamping, and the rest is given in the form of maintenance dose. Amiodaron oral tablet is initiated (600 mg/day), and is used as 200 mg/day for at least 3 months after the discharge.

Amiodaron infusion was started without discrimination in the groups of patients included in our study at the loading dose (600 mg) after cross-clamping, and maintenance Amiodaron infusion (600 mg) was initiated after the loading dose. Following decannulation, internal cardioversion was applied to patients who had AF. Patients were taken to the intensive care unit and followed up with full monitoring. Rhythm follow-up was carried out with 12-derivation electrocardiography (ECG). In the intensive care unit, patients were re-evaluated in terms of rhythm, and patients without hemodynamic problems were taken to the ward. Patients were discharged with planned anticoagulant and antiarrhythmic treatment. The ECG data and postoperative 6th month TTE follow-up results were recorded in routine follow-ups of patients after the discharge (3rd month, 6th month, and 1st year).

Statistical Analysis

The SPSS 21.0. (IBM Corp. Armonk, NY: USA. Released 2012) program was used to analyze the data obtained in the study, and $p < 0.05$ was taken statistically significant. While analyzing the study data, besides descriptive statistical methods (Mean ± standard deviation); Student t-test or Mann-Whitney U-test was used in cross-group comparisons of parameters that showed normal distribution or not in the comparison of the quantitative data; Chi-Square Test was used to compare the qualitative data.

RESULTS

There were 40 patients in Group I, and Group II included 59 patients in our study. Isolated left atrial ablation was applied to all patients. The mean age of the patients was 60.6 ± 9 years in Group I; and the mean age of the patients in Group II was 60.7 ± 9.1 (p = 0.960). Among the patients in Group I, 26 (65%) were females, and 14 (35%) were males; and in Group II, 35 (59.4%) were female, and 24 (40.6%) were male (p = 0.569). When the preoperative patient characteristics were evaluated, no statistically significant differences were detected between the groups in terms of chronic obstructive pulmonary disease, diabetes mellitus, hypertension, and coronary artery disease. The demographic characteristics of patients are presented in Table 1.

When the groups were compared in terms of preoperative EF, EF values of patients in Group II were lower than patients in Group I and statistically significant differences were found (Group I: 54 ± 7.5 vs Group II: 50.25 ± 6.1, p = 0.014). There were no statistically significant differences between the other TTE parameters that were evaluated; preoperative LA diameter and PAP values (p = 0.327 and p = 0.947; respectively) (Table 1).

Considering the surgical procedures applied to the patients; Mitral valve replacement in 48 (48.5%) patients, mitral valve replacement with aortic valve replacement in 13 (13.1%) patients, tricuspid ring annuloplasty with mitral valve replacement in 12

Table 1. Demographic and preoperative features of the patients

Variables	Group I (n = 40)	Group II (n = 59)	*p* value
Age (years)	60.6 ± 9 (40-80)	60.7 ± 9.1 (36-82)	0.960
Gender (Male/Female)	26/14	35/24	0.569
COPD	10 (25%)	15 (25.4%)	0.962
DM	7 (17.5%)	13 (22%)	0.581
HT	13 (32.5%)	14 (23.7%)	0.336
CAD	7 (17.5%)	10 (16.9%)	0.943
EF (%)	54 ± 7.5 (40-65)	50.25 ± 6.1 (35-65)	**0.014**
PAP (mmHg)	48.1 ± 9.3 (30-70)	48.2 ± 8.4 (30-70)	0.947
LA diameter (mm)	56.2 ± 6.7 (43-70)	55.1 ± 6.8 (44-77)	0.327

Data are shown as mean ± standard deviation (minimum-maximum) or number (%). COPD = Chronic obstructive pulmonary disease, DM = Diabetes mellitus, HT = Hypertension, CAD = Coronary artery disease, EF = Ejection fraction, PAP = Pulmonary artery pressure, LA = Left atrium

Table 2. Surgical procedures

Procedure	Group I (n = 40)	Group II (n = 59)	Total (n = 99)
AVR + MVR	5 (12.5%)	8 (13.5%)	13 (13.1%)
MVR	20 (50%)	28 (47.4%)	48 (48.5%)
MRA	4 (10%)	7 (11.8%)	11 (11.1%)
MVR + CABG	3 (7.5%)	3 (5.1%)	6 (6.1%)
MRA + CABG	1 (2.5%)	2 (3.4%)	3 (3%)
AVR + MRA	0	2 (3.4%)	2 (2%)
AVR + MVR + TRA	1 (2.5%)	1 (1.7%)	2 (2%)
MVR + TRA	6 (15%)	6 (10.2%)	12 (12.1%)
MVR + TRA + CABG	0	1 (1.7%)	1 (1%)
MRA + TRA	0	1 (1.7%)	1 (1%)

Data are shown as number (%). AVR = Aortic valve replacement, MVR = Mitral valve replacement, MRA = Mitral ring annuloplasty, CABG = Coronary artery bypass grafting, TRA = Tricuspid ring annuloplasty

(12.1%) patients and mitral ring annuloplasty in 11 (11.1%) patients. In addition, CABG was applied as an additional surgical procedure in 10 (10.1%) patients. Surgical procedures applied to patients are shown in Table 2.

Both groups were compared in terms of perioperative total perfusion times (TPt), cross-clamp times (CCt) and ablation times. Mean TPt was 113.5 ± 11 minutes in Group I, 109.6 ± 10.1 minutes in Group II; mean CCt was 76.8 ± 7.3 minutes in Group I and 74.1 ± 10.4 minutes in Group II. While no statistically significant difference was found between the groups in terms of TPt, CCt was significantly lower in Group II ($p = 0.101$ and $p = 0.043$; respectively). When the mean ablation times were examined, it was found that it was 13.7 ± 1 minutes in Group I and 8.6 ± 1 minutes in Group II, and this difference was statistically significant ($p < 0.001$) (Table 3).

When the operation output rhythms of the patients were evaluated, Sinus rhythm was achieved at a rate of 80% in Group I and 79.7% in Group II, and no significant difference was found ($p = 0.967$). In routine follow-ups of the groups after the discharge, 1 patient in Group I and 2 patients in Group II returned to AF rhythm during the follow-ups in the 3rd month. During the follow-ups in the 6th month, it was seen that 3 patients in Group I and 5 patients in Group II returned to sinus rhythm. In statistical terms, no statistical differences were detected between the groups compared to the 3rd and 6th month sinus rhythm rates ($p = 0.961$

Table 3. **Perioperative data**

Variables	Group I (n = 40)	Group II (n = 59)	*p* value
TPt (min)	113.5 ± 11 (95-141)	109.6 ± 10.1 (96-150)	0.101
CCt (min)	76.8 ± 7.3 (68-111)	74.1 ± 10.4 (58-115)	**0.043**
Ablation time (min)	13.7 ± 1 (12-15)	8.6 ± 1 (7-10)	**< 0.001**

Data are shown as mean ± standard deviation (minimum-maximum). TPt = Total perfusion time, CCt = Cross-clamp time

Table 4. **Early and mid-term rhythm results of the patients**

Variables	Group I (n = 40)	Group II (n = 59)	*p* value
Postoperative sinus rhythm	32 (80%)	47 (79.7%)	0.967
3rd month sinus rhythm	31 (77.5%)	45 (76.2%)	0.961
6th month sinus rhythm	34 (85%)	50 (84.7%)	0.975
1st year sinus rhythm	34 (85%)	50 (84.7%)	0.975

Data are shown as number (%).

Table 5. **Sixth month transthoracic echocardiography data of the patients**

Variables	Group I (n = 40)	Group II (n = 59)	*p* value
EF (%)	53.1 ± 6.9 (35-65)	49.5 ± 6.7 (30-60)	**0.013**
PAP (mmHg)	36.7 ± 7.3 (25-50)	37.5 ± 6.3 (25-55)	0.633
LA diameter (mm)	47.3 ± 4.6 (39-56)	46.1 ± 4.8 (38-61)	0.117

Data are shown as mean ± standard deviation (minimum-maximum). EF = Ejection fraction, PAP = Pulmonary artery pressure, LA = Left atrium

and $p = 0.975$; respectively). In the 1st year of follow-ups, it was determined that sinus rhythm continued at a rate of 85% in Group I and 84.7% in Group II, and no statistically significant difference was found ($p = 0.975$). In line with the data obtained, no return to sinus rhythm was observed after the 6th month follow-ups (Table 4).

When the 6th month TTE results were evaluated, it was seen that there were statistically weakly significant differences between the groups compared in EF values ($p = 0.013$); and there were similar values with EF values in the preoperative period. In the 6th month, no statistically significant differences were detected between the groups in terms of LA diameter and PAP values ($p = 0.633$ and $p = 0.117$, respectively) (Table 5).

DISCUSSION

AF increases mortality and morbidity at significant levels, as well as significantly impairs the quality of life of patients and imposes a significant socioeconomic burden on patients. Arrhythmia is the cause of approximately 10% of hospitalizations related to the circulatory system. Most of this is composed of AF and atrial flutter [3, 6]. In patients who undergo cardiac surgery, the achieving sinus rhythm in the postoperative period is very important for healing process [9, 10].

The groups in our study were those who underwent irrigated unipolar RF ablation and cryoablation, and all of them had pathology in the mitral valve. In the literature, the presence of AF rhythm was reported in 30-79% of patients who undergo valve surgery and the rate of spontaneous return to postoperative sinus rhythm was less than 10% [11, 12]. However, Forlani *et al.*'s [13] study argued that the return to sinus rhythm increased the survival rates of patients and reduces morbidity significantly. For this purpose, the application of ablation therapy together with mitral intervention will be beneficial in patients with persistent AF rhythm in the preoperative period.

Several complications might develop due to this

process. The complications reported so far in the literature consist of endocardial applications. Regardless of the probe used, bleeding due to esophageal damage or left atrium perforation may develop especially in left atrial RF ablation. Phrenic nerve damage may occur during cryoablation. However, cryoablation has low thrombogenicity compared with RF ablation, with a low perioperative bleeding and atrial wall perforation risk [14]. The most fatal complication in endocardial practice is esophageal damage [15]. During the circling of the right and left pulmonary vein mouths in two separate islets and the combination of these two, the esophageal may be damaged due to its neighboring localization to left atrium posterior wall. In our clinical applications, to avoid such complications and minimize the possibility of damage, the TEE probe and nasogastric catheter are removed (if any) during the ablation process, and gauze is placed in the oblique sinus. Also, attention must be paid not to intersect the left atrial circles because applying energy to the same tissue for a second time increases the risk of perforation [16]. Doll *et al*. [15] applied RF ablation to 387 patients, and reported that esophageal perforation developed in four patients (1%). Also, circumflex artery injury during Maze Operation combined with cryoablation, left main bronchial injury because of its proximity to the posterior wall of the left atrium, and pulmonary vein stenosis can also be named among related complications [17, 18]. No complications were detected in our patients in our study, including esophageal damage and left atrial wall perforation.

In the present study, it was observed that TPt was shorter in patients who underwent RF ablation compared to those who underwent cryoablation. In his study, Güden *et al*. [19] reported that the endocardial procedure lasted between 9 and 12 minutes for left atrial ablation. In our study, cryoablation lasted 12-15 minutes, and RF ablation process lasted 7-10 minutes. It was observed in our study that RF ablation process was significantly shorter, when the ablation application times were compared between both groups ($p < 0.001$). When TPt and CCt, which included ablation and surgical procedure, were compared, no statistically significant difference was found between the two groups in terms of TPt. In terms of CCt, CCt was found to be significantly shorter in the RF ablation group ($p = 0.043$).

The amputation of atrial appendices, especially the left atrial appendix, is still controversial today. However, with the risk of thromboembolic events, it is still considered as a logical approach to perform atrial amputation, or at least applying ablation to the base, then sewing it from the inside, or by inserting a pouch suture from the outside, closing the mouth completely [20]. We also think in line with this approach in our clinic, and apply internal ligation to the left atrial appendix in the framework of our surgical ablation protocol.

One important point that stands out in all studies reporting RF ablation results was that although the rates of recovering from AF after surgery were always close to 100%, a significant decrease also occurred, especially in the first week, followed by a re-increase in six months to one year. In our study, it was found that there was a return from sinus rhythm to AF rhythm in the first 3 months (1 patient in Group I, and 2 patients in Group II). After 1st year follow-up, it was found that 3 patients in cryoablation group and 5 patients in RF ablation group returned to sinus rhythm in the last nine months. This condition is explained with temporary shortening of the refractory period of atrium because of edema, inflammation and high catecholamine levels in circulation as a result of surgical trauma in the postoperative period [21]. However, AF can also be seen in early postoperative period because of advanced age, increased sympathetic activity, ischemia and electrolyte disorder, atrial factors, and other underlying cardiac diseases. This period lasts about 3 months, and patients are advised to continue their antiarrhythmic treatments. For this reason, Geidel *et al*. [22] recommended using "Amiodaron" in the first 3 months. Antiarrhythmic treatment (Amiodaron) is applied to all patients in the first 3 months of postoperative period according to the medical treatment protocol of our clinic after cryoablation and radio-frequency ablation. For this reason, six-month, even one year and above follow-up times should be considered in the evaluation of surgical treatment results of AF. In terms of clinical applications, it must also be noted that AF attacks in early postoperative period may be temporary. It should be known that the actual result will be determined in a longer period.

There are numerous studies in the literature that compare cryoablation and RF ablation methods in terms of clinical outcomes, efficacy and safety. In the case control study of Linhart *et al*. [23] with 40 pa-

tients with paroxysmal AF, they found that cryoablation and RF ablation had similar success rates. Kojodjojo *et al*. [24] compared cryoablation and RF ablation in 124 patients with persistent and paroxysmal AF. The success rate at the end of one year follow-up was reported to be 77% in cryoablation group, and 72% in RF ablation group. Kühne *et al*. [25] conducted a study and compared cryoablation and RF ablation procedures, 88% success rate was reported in cryoablation group, and 92% success rate in RF ablation group in one year follow-up. In the "Fire and Ice" study published by Kuck *et al*. [26], 762 patients who underwent cryoablation and RF ablation were followed-up in two groups for approximately 18 months, and had no significant differences when the effectiveness and reliability of the two methods were compared. Also, a large-scale meta-analysis study compiled by Hachem *et al*. [27] consisting of 247 randomized controlled trials found similar results with "Fire and Ice" study. In our study, at the end of the 1st year, 85% success rate was reported in cryoablation group, and 84.7% in RF ablation group with no statistically significant differences between the groups ($p = 0.975$). The success rates we achieved in our study showed similar results with these studies in terms of the comparison of cryoablation and RF ablation methods, and suggested that both methods are not superior to each other, and their efficacy is similar.

Limitations

The fact that the number of patients was low in our study, its retrospective design, high diversity of patients, and lack of a long follow-up period were the limitations of our study. In this respect, we believe that large-scale studies should be conducted with larger patient series and longer follow-up periods.

CONCLUSION

As a conclusion, sinus rhythm was achieved at a high rate with cryoablation and irrigated unipolar RF ablation applied to patients in preoperative AF rhythm scheduled for open heart surgery. When they were compared in terms of postoperative morbidity, mortality, rhythm control and echocardiographic values, it was observed that both methods were effective and reliable; however, at the same time they had no superiority over each other.

Authors' Contribution

Study Conception: ABT; Study Design: ABT, AAP; Supervision: YA; Funding: ABT; Materials: ABT, AAP, ME; Data Collection and/or Processing: ABT, ME, TT; Statistical Analysis and/or Data Interpretation: AAP, YA; Literature Review: ABT, ME, TT; Manuscript Preparation: ABT, AAP, TT and Critical Review: YA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2021;7(5):509-514 Original Article

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Mesenteric panniculitis: can venous anatomy and malignancy be a trigger cause?

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ABSTRACT

Objectives: To investigate whether portal vein (PV), splenic vein (SV), superior mesenteric vein (SMV) diameters and portomesenteric angle (PMA) may play a role in the etiology of mesenteric panniculitis (MP). To investigate relationship between MP and malignancy.

Methods: We included 70 patients with MP and 70 patients age, gender, sagittal abdominal diameter randomly matched control group, retrospectively.

Results: We found slightly higher PV and SMV diameter and slightly lower SV diameter in MP patients compared with control group. But these results were not statistically significant ($p_1 = 0.321$ and $p_2 = 0.147$, $p_1 = 0.540$ and $p_2 = 0.185$, $p_1 = 0.216$ and $p_2 = 0.617$, for two observers respectively). We found higher PMA in MP patients compared with control group but this difference is not statistically significant ($p_1 = 0.252$ and $p_2 = 0.366$, for two observers). Twenty three (32.9%) of 70 patients diagnosed MP have underlying malignancy while 17 (24.3%) of 70 control subject were coexisted malignancy. But this difference is not statistically significant ($p = 0.262$). Interobserver agreement was excellent in terms of SMV and SV diameters and PMA measurements (ICC were 0.927, 0.911 and 0.965 respectively), good for PV diameters (ICC was 0.884).

Conclusions: Study results show that MP is not associated with PV, SMV and PMA. Contrary to some studies, there is no relationship between MP and malignancy.

Keywords: Mesenteric panniculitis, computed tomography, portomesenteric angle, malignancy

Mesenteric panniculitis (MP) is an uncommon disease characterised by chronic nonspecific inflammatory process that affects the adipose tissue of the bowel mesentery [1]. MP has several synonyms such as retractile mesenteritis, mesenteric lipodystrophy and sclerosing mesenteritis [2, 3]. MP mostly occurs in mid to late adulthood, showing a male predominance and with a prevelence of 0.16%-2.5% [1, 4]. Histopathologically, altered adipocytes and lipid laden macrophages with mild inflammatory reaction and fibrosis were showed [5]. MP has various clinical presentations, up to a third of patients may be symptomatic [6]. MP is most frequently diagnosed incidentally by computed tomography (CT), but it also can be diagnosed with magnetic resonance imaging and abdominal sonography [6].

Although etiology of the disease is unclear, variety of conditions such as vasculitis, granulomatous disease, rheumatic disease, malignancies, trauma, pancreatitis, autoimmune disorders, ischemia and pre¬vious abdominal surgery are thought to be related to the disease. Especially relationship with malignancy

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has been extensively studied in the literature and conflicting results were reported [1-7].

It is known that prolonged venous congestion causes edema, cell atrophy, necrosis and with time fibrosis develops. Chronic venous congestion and increased hydrostatic pressure may cause venous dilatation. According to this finding we hypothesized that MP may develop due to chronic congestion and this may be associated with increased portal vein (PV), splenic vein (SV) and superior mesenteric vein (SMV) diameters. Similarly, portomesenteric angle (PMA) may also affect blood flow and venous return and may be associated with increased hydrostatic pressure and venous congestion.

This study has 3 main objectives. First, to find if there is any correlation between PV, SMV, SV diameters and MP. Second, to investigate the association between PMA and MP. And third, to determine the presence and rate of malignancy accompanying MP.

METHODS

Patients

We retrospectively examined 100 patients with diagnosis of MP who underwent CT examination in our radiology department between January 2017 and March 2020. Diagnostic criteria for the MP were described as 1) presence of a well-defined fatty mass at the root of the mesentery displacing neighboring structures 2) a higher attenuation and inhomogeneity than retroperitoneal, mesocolon or subcutaneous fat tissue; 3) containing small lymph nodes within the fatty mass; 4) a hypodense fatty halo sign that surrounding vessels and lymph nodes and 5) a hyper-attenuating pseudocapsule surrounding the mesenteric fatty mass [6]. Presence of at least 3 of the 5 typical signs required for diagnosis. All CT images were re-evaluated and diagnosis was confirmed by two abdominal radiologist seperately (MAG and RS).

Exclusion criteria were determined as lack of IV contrast administration (n = 22), presence of a tumor invading the portal vein that may affect the venous diameters (n = 4), advanced stage cirrhosis (n = 1), heavy image artefacts and technical reasons (n = 3) (Fig. 1). A total of 70 patients with diagnosis of MP included the study. For each patient with MP, one control patient without evidence of MP was selected. Control groups were matched for age, gender, sagittal abdominal diameter (SAD) and CT protocol. Our institutional ethical committee approved the study protocol (decision number: 08/147, date:11.06.2020). Details of the identified clinical data are shown in Table 1.

Imaging Protocol

All CT images obtained using a 16-section multi detector CT system (Aquilion 16; Toshiba Medical Systems, Japan). Abdominopelvic CT was performed in the craniocaudal direction from the level above the diaphragm to the symphysis pubis. The patients were instructed to hold their breath with tidal inspiration during examination. CT parameters were as follows: 32×1 mm collimation, 1.25 pitch, 0.5 seconds rotation time, 5 mm reconstructed section thickness and 5 mm intersection gap, 120 kV tube voltage, 250 mA tube current-time product. The axial section data were reconstructed at a thickness of 5 mm with 5-mm increments and a thickness of 2 mm with 1-mm increments. Maximum intensity projection (MIP) images were reconstructed by using coronal reformatted images with

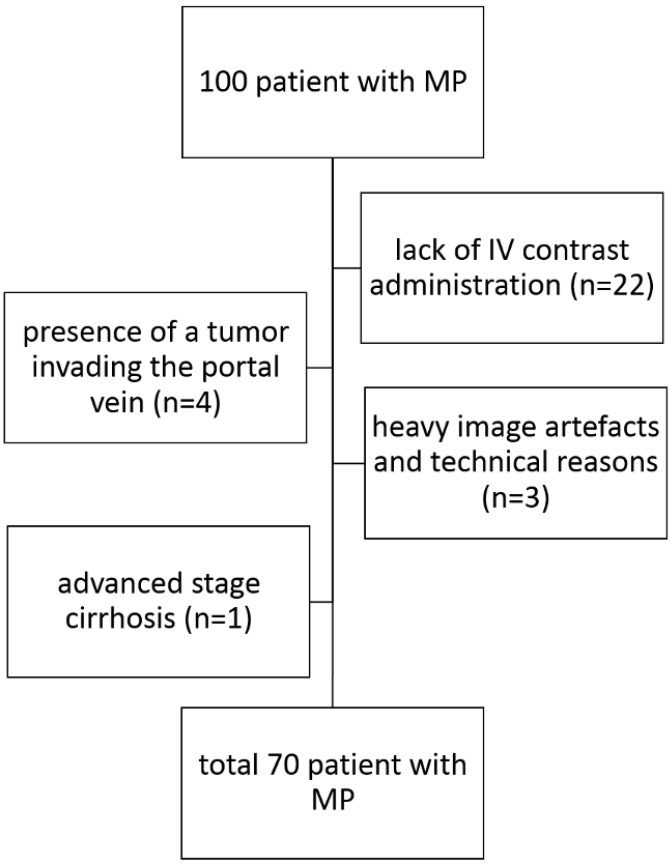

Fig. 1. Diagram showing the exclusion criteria of patients for study. MP = mesenteric panniculitis

Table 1. Baseline patient characteristics

	MP patients (n = 70)	Control group (n = 70)	*p* value
Age, years	57.7 ±1 0.9	57.4 ± 13.1	0.905
Gender (female/male)	33/37	33/37	1
SAD, mm (mean ± SD)	255 ± 21.6	254.9 ± 31.6	0.988

MP = mesenteric panniculitis, SAD = sagittal abdominal diameter, SD = standart deviation

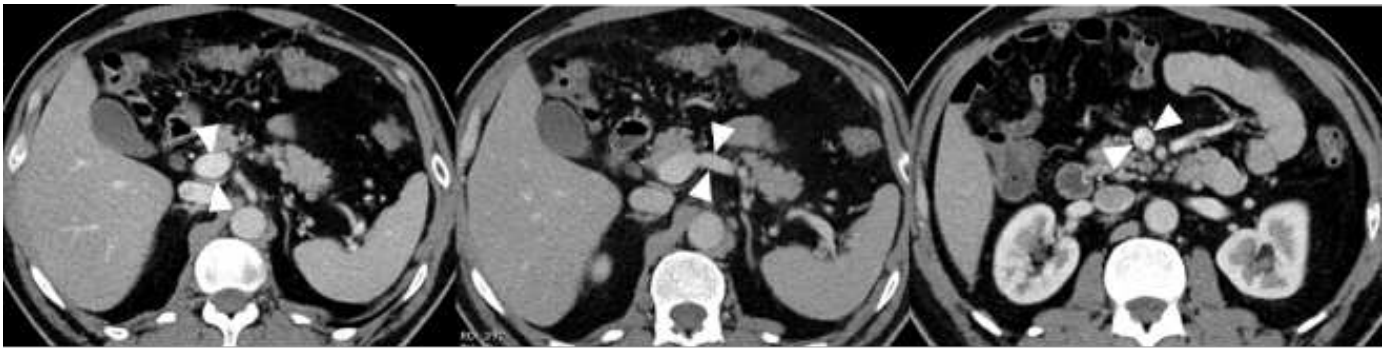

Fig. 2. Contrast enhanced axial CT image shows measurements for portal vein, splenic vein and superior mesenteric vein (arrowheads).

64 slices on the our PACS system (FUJIFILM Medical Systems USA). Seventy milligrams of intravenous contrast material (iohexol; Omnipaque 350, GE Healthcare, Cork, Ireland) was administered in all patients at a rate of 3 ml/s with a power injector. CT was started 70 seconds after start of contrast material injection.

CT Measurements

PV, SMV and SV diameters were measured outer wall-to-outer wall on axial images on the portal venous phase (Fig. 2). All measurements were performed at the point of 1 cm distal (SMV and splenic vein) and proximal (portal vein) to the portal confluence. MIP images were used for PMA measurements. A line parallel to the main portal vein was drawn from central section of the portal vein at the branching level. A second line was drawn from the central point of the portal confluence to the distal cental point of mesenteric vein at the branching level. Angle between the two lines was measured and recorded (Fig. 3). All images were evaluated by two abdominal radiologist having more than 5 years of experience in abdominal imaging (MAG and RS).

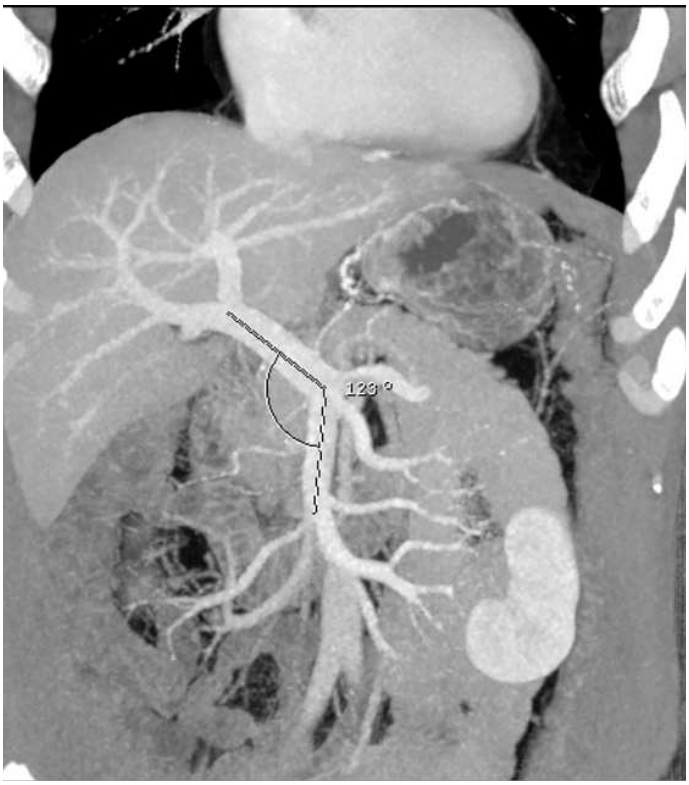

Fig. 3. Contrast enhanced, coronal CT maximum intensity projection image shows measurement of portomesenteric angle.

Statistical Analysis

Statistical analysis was performed by the IBM SPSS Statistics 20.0 statistical software (Armonk, NY: IBM Corp.). Kolmogorov-Smirnov test was used for normality. Mean ± standard deviations presented as descriptive statistics. In the comparisons of the PV, SMV, SV diameters and PMA in the MP and control group the independent samples t-test was used. Pearson Chi-Squared test was used to determine the presence of concomitant malignancy for two groups. A p value below 0.05 was accepted as statistical significance. Interobserver agreement for evaluation of all measurements was performed. The intraclass correlation coefficient (ICC) was calculated to assess the degree of agreement between two observers for PV, SMV and SV diameters and PMA measurements.

RESULTS

A total of 70 patients with diagnosed MP (33 females, 47.1%: 37 males, 52.9%) and age, gender and abdominal diameter matched 70 control group (33 females, 47.1%: 37 females, 52.9%) were included the study. The mean age of the samples were 57.7 ± 10.8 years and 57.4 ± 13.1 years, for MP and control group, respectively. The SADs of the samples were 255 ± 21.6 mm and 254.9 ± 31.6 mm, for MP and control group respectively. As populations were matched, there was no difference in gender, age and SAD between patients and control group.

There were no statistically significant differences between MP and control group in terms of PV diameter (p_1 = 0.321 and p_2 = 0.147, for two observers), SMV diameter (p_1 = 0.540 and p_2 = 0.185, for two observers), SV diameter (p_1= 0.216 and p_2 = 0.617, for two observers) and PMA (p_1 = 0.252 and p_2 = 0.366, for two observers). These results are shown in Table 2.

Twenty three (32.9%) of 70 patients diagnosed MP have underlying malignancy while 17 (24.3%) of 70 control subject were coexisted malignancy. But this difference is not statistically significant (p = 0.262). These results are summarized in Table 2.

Underlying malignancies with MP were colorectal (n = 7), breast (n = 3), lymphoma (n = 2), gastric (n = 2), renal (n = 2), prostatic (n = 1), cervical (n = 1), endometrial (n = 1), pancreas (n = 1), lung (n = 1), bladder (n = 1) and testis (n = 1). Remaining 47 of 70 (67.1%) patient diagnosed MP had no underlying malignancy.

Interobserver agreement was excellent in terms of

Table 2. Comparison of PV, SV, SMV diameter, PMA and concomitant malignancy rates of two group

	MP patients (n = 70)		Control group (n = 70)		*p* value		ICC
	Observer 1	Observer 2	Observer 1	Observer 2	Observer 1	Observer 2	
PV diameter, mm (mean ± SD)	140.6 ± 17.9	139.6 ± 16.9	137.8 ± 14.1	135.6 ± 15.6	0.321	0.147	0.884
SV diameter, mm (mean ± SD)	88.7 ± 14.5	89.3±13.7	91.9 ± 15.4	90.6 ± 16.8	0.216	0.617	0.911
SMV diameter, mm (mean ± SD)	121.5 ± 17.5	120.6 ± 17.4	116.2 ± 14.3	116.9 ± 15.4	0.540	0.185	0.927
PMA, degree (mean ± SD)	131.1 ± 12.6	130.9 ± 13.5	128.2 ± 17.3	128.4 ± 18.5	0.252	0.366	0.965
Concomitant malignancy, n (%)	23 (32.9%)		17 (24.3%)		0.262		

ICC = intraclass correlation coefficient, MP = mesenteric panniculitis, SD = standart deviation, PMA = portomeseneric angle, PV = portal vein, SMV = superior mesenteric vein, SV = splenic vein

lesion SMV and SV diameters and PMA measurements (ICC were 0.927, 0.911 and 0.965 respectively), good for PV diameters (ICC was 0.884).

DISCUSSION

MP refers to CT appearance of increased density of the intestinal mesentery by chronic nonspecific inflammation and fibrosis. Although the exact etiology is still unknown, various mechanisms underlying MP have been discussed in the literature such as previous abdominal surgery, trauma, autoimmunity, ischemic injury and paraneoplastic syndrome [7]. Especially its relationship with malignancy has been extensively studied and there are contradictory results in the literature. While some studies showed increased risk of malignancy [1, 3, 8-10], some studies have reported contrary results [6, 7, 11]. In the current study, 32.9% of patients diagnosed with MP had underlying malignancy while 24.3% of control subjects had underlying malignancy. Although higher rate of malignancies have been found in MP patients, this difference is not statistically significant ($p = 0.262$). Pathophysiological process between mesenteric panniculitis and the development of malignancy is also unknown. Kipfer *et al*. [5] suggested that mesenteric panniculitis is a nonspecific response to an underlying abdominal malignancy. However, it was reported that extraabdominal malignancies can also accompany MP [1]. Four of 23 patients had extraabdominal malignancy (3 breast carcinoma and 1 lung carcinoma) in our study.

Conditions that may affect the vascular supply of the mesentery such as abdominal surgery or mesenteric thrombosis were also associated with mesenteric panniculitis [12, 13]. Seo *et al*. [3] found dilated vessels within the misty mesentery in 27 (93%) of the 29 patients with MP. More of them had venous dilatation (23 of 27 patients). They were in the opinion that venous dilatation resulted from hydrostatic pressure or from vascular compression of the vein draining the mesentery [3]. In the lights of these findings we thought that there may be a correlation between PV, SV, SMV diameters and MP. PMA may also affect the venous return that may causes increased hydrostatic pressure and congestion. We found slightly higher PV and SMV diameter in MP patients compared with control group. However these differences are not statistically significant. On the contrary, SV diameters were slightly lower in MP patients compared with control group. Additionally, we found slightly higher PMA in MP patients compared with control group but its not significant. Especially, dilated SMV, that draining the mesentery, may be associated with increased congestion and fibrosis. Higher PMA may cause decreased venous return and increased hydrostatic pressure, congestion and fibrosis.

Our study has some limitations. First one is the retrospective design of the study. Second, lack of histopathologic confirmation of the MP. Diagnosis of MP was made based on diagnostic CT criteria. None of our patients underwent histopathological examination because histopathological examination is unnecessary in most cases. Third, MP and control groups were consisted of selected patients with known diagnosis. This may cause bias in measurements.

CONCLUSION

As to our knowledge this is the first study to investigate venous diameter in MP. Our study results showed slightly increased PV, SMV diameter and PMA in MP however it is not significant with exellent and good interobserver agreement. We also investigated association between malignancy and MP. Although higher rate of malignancies have been found in MP patients, contrary to some studies this difference is not statistically significant. With these results, we think that venous anatomy and precense of malignancy are not included in the etiology of MP. Larger studies are needed to prove the exact etiology of MP.

Authors' Contribution

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Conflict of interest

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The relationship between disease prognosis and serum calcium and corrected calcium levels in COVID-19 patients

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ABSTRACT

Objectives: The present study aimed to evaluate whether low serum calcium (Ca) and corrected calcium (cCa) levels could predict disease prognosis and mortality in patients with COVID-19.

Methods: In this study, we retrospectively enrolled 206 eligible patients with COVID-19, diagnosed at Turkey Kanuni Sultan Süleyman Training and Research Hospital between March 12, 2020 and June 15, 2020.

Results: Serum Ca level was 8.8 ± 0.57 mg/dL and the serum cCa level was 8.99 ± 0.53 in all patients. The patients were divided into two groups, such as hypocalcemic and non-hypocalcemic patients. We observed that serum Ca levels of patients who died were significantly lower than that of surviving patients. A significant negative correlation was found between serum cCa level and albumin level. A significant positive correlation was found between serum cCa level and C-reactive protein, lactate dehydrogenase, ferritin, procalcitonin, troponin, CURB-65 score, and quick Sepsis-related Organ Failure Assessment (q-SOFA) score. Univariate logistic regression analysis revealed that age, respiratory rate, saturation, heart rate, lymphocyte, serum calcium, D-dimer, CURB-65 score, and q-SOFA score were independent predictors of high-risk group of mortality.

Conclusions: This study confirms that the severity of COVID-19 is associated with lower concentrations of serum Ca. The cCa levels were associated with certain prognostic factors. Serum Ca and cCa levels could be an early and helpful marker to improve management of patients with COVID-19. We recommend evaluation of calcium in patients on initial presentation and serial monitoring during hospitalization in order to perform timely and appropriate corrective actions.

Keywords: COVID-19, mortality, calcium, corrected calcium, hypocalcemia

Coronavirus disease-19 (COVID-19), which was first manifested as atypical pneumonia cases in Wuhan, the capital of Hubei province of China in December 2019, was later found to be a new type of coronavirus. The COVID-19 disease is termed SARS-CoV-2 because its etiology resembles the severe acute respiratory syndrome coronavirus (SARS-CoV). COVID-19 disease continues to be a major health con-

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cern throughout the world, including Turkey [1, 2]. Although, COVID-19 disease can usually be manifested as an asymptomatic or mild disease, its clinical course can be severe in some cases. The severe clinical course can be predicted in patients with certain clinical features; however, this cannot be done with absolute certainty. Advanced age, male gender, presence of comorbid diseases (especially hypertension, diabetes mellitus, and coronary artery disease), obesity, hypotension, tachypnea, hypoxia, lymphopenia, thrombocytopenia, hypoalbuminemia, impaired renal function, high levels of C-reactive protein (CRP), D-dimer, procalcitonin, interleukin-6 (IL-6), ferritin, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and the presence of ground glass opacity on tomography have been reported to be poor prognostic factors for the disease [1, 3-11].

SARS-CoV-2 binds to the angiotensin-converting enzyme (ACE)-2 receptor through the S protein and enters the respiratory tract and causes cell damage through cytopathic effect and cytokine release [12]. Calcium (Ca2+) is an important molecule in viral infections for the formation of the virus structure, entry of the virus into the cell, gene expression, virion maturation, and release [13]. In vitro experimental studies and studies on animal models infected with SARS-CoV have demonstrated that the SARS-CoV E gene encodes a small transmembrane protein that is highly expressed during infection and has ion channel activity. It has been reported that these ion channels are permeable to calcium and activate inflammatory pathways through intracellular calcium homeostasis changes, disrupt the host cell system, and facilitate the replication of the virus [14-16]. The similarity between SARS-CoV-2 and SARS-CoV genomes suggests that a similar mechanism may be effective.

Serum Ca level abnormalities are common in hospitalized patients and are known to be associated with mortality [17]. While some studies reported higher mortality in hypocalcemic cases, other studies reported that hypercalcemic cases had higher mortality rates [18, 19]. However, there are no studies that have actually studied this hypothesis in patients with COVID-19. The fact that the calcium molecule plays an active role in the host cell–virus interactions prompt us to think that the differences in calcium levels can cause differences in the effects of the virus on the host cell and can thereby affect the course of the disease. Therefore, this study aimed to investigate the factors affecting the relationship between disease prognosis and mortality with serum Ca and corrected calcium (cCa) levels in patients with COVID-19.

METHODS

Study Design

The protocol of the current study was approved by the ethics committee of Health Sciences University, Kanuni Sultan Süleyman Research and Training Hospital (No: KAEK/2020.06.114). This retrospective study was conducted in the Health Sciences University, Kanuni Sultan Suleyman Research and Training Hospital, Department of Internal Medicine. We enrolled 206 patients diagnosed with COVID-19 between March 12, 2020 and June 15, 2020 in this study.

Patient Characteristics and Data Collection

This retrospective cross-sectional study was carried out utilizing the medical records of patients who were hospitalized in isolated wards with the diagnosis of COVID-19 in a tertiary education and research hospital. In this study, we included patients with positive SARS-CoV-2 RNA detection in throat swab samples and who were diagnosed with COVID-19 according to the World Health Organization (WHO) guidelines. All patients were of Turkish descent.

We recorded the treatments received by the patients, duration of hospitalization, and the disease outcome (survival or nonsurvival). Demographic data of the patients (age, gender, hospital admission complaints, and comorbid diseases) and vital signs at the time of admission (presence of fever, heart rate, percentage of oxygen saturation on room air, and minute-respiration rate) were obtained from the patient's medical record. Laboratory data [complete blood count (CBC), CRP, creatinine, ALT, AST, albumin, calcium, LDH, D-dimer, fibrinogen, ferritin, procalcitonin (PCT), and troponin] and imaging analysis of the patients at the time of admission (presence of lung involvement on chest tomography; and if there is involvement, whether it is unilateral or bilateral and presence of consolidation and ground glass appearance) were obtained from the medical records.

Patients under the age of 18, patients whose serum

Ca level was not checked at the time of hospital admission, pregnant women, breastfeeding women, patients with comorbidities that may affect serum calcium levels (such as primary hyperparathyroidism, hypoparathyroidism, malignancy, multiple myeloma, osteoporosis, granulomatous diseases, and pancreatitis), and patients who were on medications within the last 1 month that could affect serum calcium levels (such as lithium, vitamin D, thiazide diuretics, and bisphosphonates) were not included in this study.

The cCa level (mg/dL) was calculated using the following formula: measured serum Ca level (mg/dL) + 0.8 × [(4-serum albumin (g/dL)]. Patients with serum Ca level or serum cCa level < 8.5 mg/dL were defined as the "hypocalcemic group," and patients with serum Ca level or serum cCa level ≥8.5 mg/dL were defined as the "non-hypocalcemic group."

The CURB-65 score was calculated by considering 1 point for each of the following conditions: confusion, respiratory rate ≥ 30/min, systolic blood pressure < 90 mmHg or diastolic blood pressure < 60 mmHg, BUN >19 mg/dL, and age ≥ 65.

q-SOFA score was calculated by considering 1 point for each of the following parameters: abnormal state of consciousness, respiratory rate ≥ 22/min, and systolic blood pressure ≤ 100 mmHg.

Samples were tested using the WHO recommendations and national guidelines [20].

Laboratory Procedures

Blood samples were obtained prior to treatment and collected into standardized tubes containing EDTA for analysis of CBC parameters and standardized tubes without any anticoagulant for the biochemical analysis. Serum samples that were obtained after centrifugation at 2500 g for 5 min were used directly for the measurements of biochemical parameters.

CBC was analyzed by a Sysmex XT 1800i device (ROCHE-2011, Kobe, Japan). Ca and other biochemical parameters were analyzed by a COBAS 8000 device (ROCHE-2007, Tokyo, Japan).

CRP analysis was performed using the immunoturbidimetric method (ROCHE DIAGNOSTICS HITACHI, Tokyo, Japan).

Plasma fibrinogen was measured by using Clauss method using Fibrintimer II coagulometer and Multifibren U kit (Siemens Healthcare Diagnostics, Germany).

D-dimer analysis was carried out on plasma collected into 3.2% buffered sodium citrate blood tubes (Becton Dickinson, Franklin Lakes, NJ, USA), using Stago coagulation analyzers STA compact Max 3 (Stago, Asnières-sur-Seine, France) and proprietary reagents by immunoturbidimetric method.

Ferritin, PCT, and troponin were measured in VIDAS (BioMerieux Inc. France)) device by electrochemiluminescence immunoassay (ECLIA) method.

Oxygen saturation was measured every 4 hours using a digital saturometer. Oxygen flow was calculated to obtain oxygen saturation between 90% and 94%.

Computed Tomography (CT)

We used a multidetector CT scanner (Toshiba Aquilon; Toshiba, Inc., Tokyo, Japan) with the following parameters: tube voltage, 120 kV; tube current, 110 mAs (automatic adjustment); rotation time, 0.5 second; section thickness, 0.75 mm; collimation, 0.6 mm; pitch, 1; matrix, 512 × 512; and inspiration breath hold. Axial, sagittal, and coronal reformatted images were created with a slice thickness of 3-mm.

Analysis of RT-PCR Test

SARS-CoV-2 was detected by real time polymerase chain reaction method in oropharyngeal/nasopharyngeal swab samples.

Statistical Analysis

SPSS 21.0 program was used for data analysis. Nominal variables were expressed as number and percentage, whereas numeric variables were represented as mean ± standard deviation or median. Kolmogorov–Smirnov test was performed to determine whether the continuous variables were normally or non-normally distributed. Normally distributed independent continuous variables were compared with the independent t-test, whereas non-normally distributed independent continuous variables were compared with the Mann–Whitney U test among the groups. A univariate regression analysis was performed to observe the effects of possible parameters on mortality. In order to evaluate the relationship between serum Ca level and mortality, we determined a cut-off value for serum Ca level by the receiver operating characteristic (ROC) analysis. A two-tailed p value of < 0.05 was considered to be statistically significant. In the power

analysis made through the GPower 3.1.9.4 program, it has been determined that the achieved power value (1-β err prob) of the study is 1.

Table 1. Demographic data of patients

Gender	n	%
Male	113	54.9
Female	93	45.1
Complaint of admission time		
Cough	148	71.8
Fever	114	55.3
Weakness	96	45.1
Shortness of breath	88	42.7
Myalgia	36	17.5
Sore throat	15	7.3
Headache	9	4.4
Diarrhea	5	2.4
Treatment options		
Hydroxychloroquine	205	99.5
Azithromycin	195	94.7
Oseltamivir	120	58.3
Favipiravir	27	13.1
Additional antibiotic	40	19.4
Intravenous fluid replacement	34	16.5
Oxygen support	83	40.3
Enoxaparin sodium	126	61.2
Comorbid diseases		
Hypertension	73	35.4
Type-2 Diabetes mellitus	43	20.9
Chronic obstructive pulmonary disease	25	12.1
Coronary artery disease	25	12.1
Immunological disease	5	2.4
Cerebrovascular disease	3	1.5
Tomography findings		
Unilateral involvement	25	12.1
Bilateral involvement	158	76.7
No pulmonary involvement	23	11.2
Ground glass opacity	175	85
Presence of consolidation	105	51
Outcome		
Recovered	194	94.2
Died	12	5.8

RESULTS

In this study we included 206 patients (113 males, 93 females) with a mean age of 55.1 ± 15.7 years (range: 19-92 years). The demographic data of the patients are presented in Table 1.

The most common complaint of these patients was cough, the next common complaint being high fever. The most common comorbid diseases were determined as hypertension, type-2 diabetes mellitus, obstructive pulmonary diseases, and coronary artery disease. The number of patients with at least 1 comorbid disease was 104 (50.5%), the number of patients with at least 2 comorbid diseases was 60 (29.1%), whereas the number of patients with 3 or more comorbid diseases was 19 (9.2%). Chest tomography demonstrated bilateral lung involvement (76.7%) and ground glass appearance (85%) in majority of the patients, consolidation (51%) was observed in nearly half of the cases, whereas 23 patients demonstrated no signs of pneumonia. Most of the patients received hydroxychloroquine and azithromycin therapy, and more than half of the patients also received enoxaparin sodium and oseltamivir. The mean duration of hospitalization of the patients was 7.1 ± 3.3 days (2-19 days). Twelve patients (5.8%) succumbed to the disease.

When all patients were taken into consideration, serum Ca level was found to be 8.8 ± 0.57 mg/dL and serum Ca level was found to be 8.99 ± 0.53 mg/dL. The results obtained when the patients were grouped and compared as hypocalcemic and nonhypocalcemic are presented in Table 2.

The serum Ca levels of patients who died were significantly lower than those of patients who survived (8.4 ± 0.64 vs. 8.83 ± 0.56, $p = 0.013$), but there was no significant difference between serum cCa levels (9.08 ± 0.6 vs. 8.98 ± 0.53, $p = 0.544$). No significant difference was found between serum Ca levels of patients with and without pneumonic infiltration (8.80 ± 0.55 vs. 8.83 ± 0.71, $p = 0.844$), with at least one comorbid disease or no comorbid disease (8.85 ± 0.60 vs. 8.75 ± 0.54, $p = 0.188$), and who were using ACE inhibitors/angiotensin II receptor blockers or who did not use these drugs (8.93 ± 0.64 vs. 8.77 ± 0.55, $p = 0.140$).

The results obtained in the univariate regression analysis for the parameters predicted to have an effect

Table 2. Comparison of the patients in terms of serum calcium and corrected serum calcium levels

	Serum calcium		*p* value	Corrected serum calcium		*p* value
	< 8.5mg/dL (n = 57)	≥ 8.5mg/dL (n = 149)		< 8.5mg/dL (n = 42)	≥ 8.5mg/dL (n = 164)	
Age (year)	55.01 ± 15.91	55.2 ± 15.78	0.94*	52.02 ± 15.81	55.95 ± 15.72	0.104**
Sex (F/M)	22/35	71/78	0.275#	17/25	76/88	0.603#
Hospitalization duration (day)	7.59 ± 3.68	7.03 ± 3.28	0.32**	7.19 ± 3.46	7.18 ± 3.39	0.922**
Respiratory rate (min)	19.64 ± 2.87	19.48 ± 2.62	0.956**	19.66 ± 2.97	19.49 ± 2.61	0.897**
Heart rate (min)	89.43 ± 11.87	85.47 ± 9.88	0.016**	87.69 ± 12.06	86.28 ± 10.19	0.617**
Saturation (%)	92.05 ± 10.93	93.81 ± 5.65	0.569**	92.57 ± 12.28	93.51 ± 5.72	0.43**
Calcium (mg/dL)	8.16 ± 0.29	9.05 ± 0.45	< 0.001*	8.15 ± 0.34	8.97 ± 0.49	< 0.001*
Corrected serum calcium (mg/dL)	8.56 ± 0.43	9.15 ± 0.47	< 0.001*	8.36 ± 0.32	9.15 ± 0.45	< 0.001*
WBC (103µ/L)	6.31 ± 2.66	7.04 ± 3.07	0.093**	5.65 ± 2.32	7.14 ± 3.06	0.001**
Hb (g/dL)	13.74 ± 7.97	13.18 ± 1.64	0.297**	12.76 ± 2	13.48 ± 4.82	0.368**
Neutrophil (103µ/L)	4.31 ± 2.19	4.92 ± 4.91	0.529**	3.72 ± 1.78	5.01 ± 4.74	0.017**
Lymphocyte (103µ/L)	1.335 ± 0.84	1.77 ± 1.39	0.001**	1,39±0,73	1.72±1.37	0.087**
Platelet, (103µ/L)	213.63 ± 80.34	258.45 ± 100.8	0.001**	199.92 ± 54.69	257.9 ± 102.5	< 0.001**
CRP (mg/L)	65.48 ± 54.67	45.35 ± 53.28	0.001**	51.32 ± 42.98	50.82 ± 56.94	0.251**
Creatinine (mg/dL)	1.33 ± 1.34	0.88 ± 0.32	0.008**	1.36 ± 1.52	0.91 ± 0.37	0.189**
ALT (U/L)	50.84 ± 173.31	32.21 ± 26.64	0.692**	27.19 ± 17.7	39.97 ± 104.6	0.326**
AST, (U/L)	47.07 ± 120	30.85 ± 21.04	0.07**	31.73 ± 12.90	36.26 ± 73.3	0.112**
Albumin, (g/L)	3.49 ± 0.5	3.87 ± 0.43	< 0.001*	3.74 ± 0.46	3.73 ± 0.49	0.698*
LDH (U/L)	312.8 ± 106.17	271.9 ± 90.87	0.01*	299.7 ± 92.9	279.8 ± 98.17	0.263*
D-dimer (mg/L)	1.81 ± 5.22	1.17 ± 2.84	0.637**	1.04 ± 2.13	1.42 ± 3.93	0.409**
Fibrinogen (mg/dL)	428.2 ± 136.3	420.1 ± 150.9	0.378**	388.1 ± 75.7	431.4 ± 158.2	0.559**
Ferritin (mg/mL)	664.2 ± 177.8	334.1 ± 376.9	0.016**	724.69 ± 212.8	356.1 ± 390.8	0.277**
Procalcitonin (mg/mL)	0.38 ± 1.04	0.08 ± 0.15	0.007**	0.20 ± 0.53	0.15 ± 0.59	0.869**
Troponin (mg/mL)	0.024 ± 0.042	0.016 ± 0.025	0.498**	0.020 ± 0.035	0.017 ± 0.03	0.881**
CURB-65 score	0.85 ± 0.93	0.51 ± 0.82	0.011*	0.64 ± 0.9	0.6 ± 0.86	0.795*
q-SOFA score	0.66 ± 0.8	0.45 ± 0.78	0.089*	0.5 ± 0.7	0.51 ± 0.81	0.894*

*: Student T test, **: Mann Whitney U test, #: Ki kare test

WBC = White Blood Cell, Hgb = Hemoglobin, CRP = C-reactive protein, ALT = Alanine transferasis, AST = Aspartat transferasis, LDH = Lactat dehidrogenasis, q-SOFA = Quick Sepsis-related Organ Failure Assessment

on mortality are presented in Table 3. When incorporated into the univariate analysis, age, respiratory rate, saturation, heart rate, lymphocyte, serum Ca, D-dimer, CURB-65 score, and q-SOFA score remained as significant predictors of mortality. CRP, ferritin, corrected calcium and PCT were not associated with mortality. In the correlation analysis, a positive correlation was observed between serum Ca level and lymphocyte (r: 0.14, $p = 0.045$), platelet (r: 0.262, $p < 0.001$), and albumin (r: 0.436, $p < 0.001$); and a negative correlation was found between serum Ca level and CRP (r: −0.291, $p < 0.001$), LDH (r: −0.335, $p < 0.001$), creatinine (r: −0.219, $p = 0.002$), ferritin (r: −0.305, $p < 0.001$), procalcitonin (r: −0.237, $p = 0.001$), and CURB-65 score (r: −0.148, $p = 0.033$). There was a positive correlation between serum cCa level and CRP (r: 0.23, $p = 0.003$), LDH (r: 0.252, $p = 0.002$), ferritin (r: 0.304, $p < 0.001$), procalcitonin (r: 0.241, $p = 0.002$), troponin (r: 0.381, $p < 0.001$), CURB-65 score (r: 0.205, $p = 0.008$), and q-SOFA score (r: 0.176, $p = 0.024$). On the contrary, there was a negative correlation between cCa level and albumin level (r: −0.303, $p < 0.001$).

Using the ROC analysis, the cut-off value of

Table 3. Univariate regression analysis for parameters predicted to have an effect on mortality

	Univariate		
Variable	**OR**	**95% CI**	***p* value**
Age	0.927	0.885-0.970	**0.001**
Respiratory rate, min	0.672	0.549-0.822	**< 0.001**
Saturation, %	1.049	1.005-1.094	**0.028**
Heart rate, min	0.932	0.887-0.980	**0.006**
Lymphocyte, 103µ/L	3.064	1.038-9.046	0.043
CRP, mg/mL	0.992	0.984-1.001	0.07
Serum calcium, mg/dL	3.960	1.328-11.806	**0.014**
Corrected serum calcium, mg/dL	0.719	0.250-2.068	0.541
D-dimer, mg/L	0.871	0.83-0.970	**0.012**
Ferritin, mg/mL	0.999	0.998-1.000	0.251
Procalcitonin, mg/mL	0.64	0.378-1.096	0.105
CURB-65 score	0.222	0.111-0.447	**< 0.001**
q-SOFA score	0.094	0.032-0.275	**< 0.001**

serum Ca level to examine the relationship between serum Ca level and mortality was determined as > 8.3 mg/dL. As presented in fig.1, mortality was significantly lower if serum Ca was > 8.3 mg/dL ($p = 0.028$, AUC: 0.681, sensitivity: 79.9%, specificity: 50%).

DISCUSSION

Since there is a high prevalence of hypocalcemia in COVID-19 patients and also due to the fact that hypocalcemia helps in predicting the need for hospitalization, it is recommended that Ca should always be evaluated initially during hospitalization to identify more severe patients [21-23]. We observed that serum Ca levels of the patients who died were significantly lower than that of the patients who survived. Furthermore, there was a significantly lower mortality in patients with serum Ca > 8.3 mg/dL (sensitivity: 79.9%, specificity: 50%) according to the ROC analysis. This study revealed that serum Ca level is a good prognostic parameter in COVID-19 patients and is an independent determinant for mortality. Patients with hypocalcemia have worse prognostic parameters. Moreover, cCa levels were associated with certain prognostic factors.

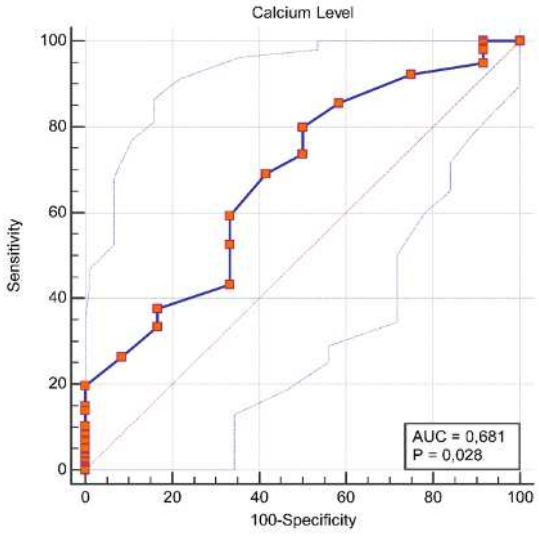

Fig. 1. ROC analysis for serum calcium.

Sun *et al*. [21] reported that the prognostic parameters of patients with COVID-19 with hypocalcemia are worse and the rates of organ failure, septic shock, and mortality are higher. In addition, they stated that the serum Ca level was associated with the severity and prognosis of the disease. In the same study, it was reported that there was a positive correlation between serum Ca and lymphocyte, albumin and SpO2; and a negative correlation between serum Ca, CRP, and D-dimer and that serum Ca levels of patients who died were lower [21]. In another study which included 585 patients who visited the emergency department with suspicion of COVID-19, patients who were diagnosed with COVID-19 and who were not diagnosed with

COVID-19 were evaluated for serum total and ionized Ca, and it was reported that serum total Ca and ionized Ca levels were lower in COVID-19 patients. It has been stated that the total Ca and ionized Ca levels decrease in COVID-19 patients as the age increases, and these values are lower in the male gender [22]. In a retrospective cohort study, it was reported that a high rate of hypocalcemia was detected in patients with COVID-19 at the time of presentation, and this was more common in male and patients with advanced age [23]. In the same study, it was stated that serum Ca levels of patients requiring hospitalization were lower than those not requiring hospitalization, and that serum Ca levels after hospitalization were closely associated with both, death and transfer to the intensive care. Bossoni *et al*. [24] reported severe hypocalcemia in a woman with COVID-19 disease who had underwent thyroidectomy and suggested Ca evaluation and monitoring in all hospitalized patients with COVID-19 infection. Another study reported that calcium, sodium, and potassium concentrations were significantly lower in patients with severe COVID-19 [25]. Cao *et al*. [26] observed that 65.4% of patients with COVID-19 had decreased serum Ca levels. Compared with the non-intensive care unit (ICU) patients, the patients admitted to the ICU were more likely to have low serum calcium (100% vs 61.4%). According to their results, as the Ca levels decreased, the severity of the disease increased. Ca2+ levels and/or Ca2+ channels may play a role in endocytosis and infection of SARS-Cov-2. Further studies are warranted to characterize the functional importance of this potential pathway [26].

However, there are conflicting results on the studies of calcium [18, 19]. Additional clinical information is required to interpret these abnormalities, including fluid status, serum albumin, and ionized calcium concentrations. Therefore, we also evaluated the serum cCa levels in this study. In the present study, serum Ca levels of the patients who died were significantly lower than that of patients who survived. Low serum Ca levels in our study are probably related to the high prevalence of hypovitaminosis D in Turkey, and this may be a predisposing factor in our study population [27]. In the correlation analysis, there was a weak/moderate positive correlation between serum calcium level and lymphocyte, platelet and albumin; and there was a significant weak/moderate negative correlation between serum calcium level and CRP, LDH, creatinine, ferritin, PCT, and CURB-65 score. However, we could not find any significance in this regard with respect to cCa levels. Khamis *et al*. [28] found a relationship between low cCa level and high mortality in patients with COVID-19 who were hospitalized. A total of 38% of the hospitalized patients were admitted to the ICU. This difference may be due to the fact that there were no patients in our sample who were hospitalized in the ICU. On the other hand, in our study, a significant negative correlation was observed between serum cCa level and albumin level; and a weak/moderate positive correlation was found between serum cCa level and CRP, LDH, ferritin, PCT, troponin, CURB-65 score, and q-SOFA score. These results show that cCa levels are associated with certain prognostic factors.

In the present study, univariate logistic regression analysis revealed that age, respiratory rate, saturation, heart rate, lymphocyte, serum Ca, D-dimer, CURB-65 score, and q-SOFA score were independent predictors of high-risk group of mortality. These results demonstrate that there is a higher risk of hypocalcemia associated with COVID-19 disease. As in other studies, our study also showed that advanced age, respiratory rate, saturation, heart rate, lymphocyte, serum Ca, D-dimer, CURB-65 score, and q-SOFA score were risk factors for mortality in patients with COVID-19 [4, 29-33]. In a retrospective cohort study, Zhou *et al*. [4] identified several risk factors of death in adults in Wuhan who were hospitalized with COVID-19. Similar to our results, in particular, advanced age, D-dimer levels >1 μg/mL, and higher SOFA score on admission were associated with higher odds of in-hospital mortality. Additionally, elevated levels of blood IL-6, high-sensitivity cardiac troponin I, LDH, and lymphopenia were more common in severe COVID-19 patients.

CONCLUSION

In conclusion, the results of this study reveal that serum Ca level in COVID-19 patients is a good prognostic parameter and an independent predictor for mortality, hypocalcemic patients have worse prognostic parameters, and there is a moderate/good correlation between serum Ca level and other parameters

previously reported as prognostic factors for COVID-19. Our results indicate that Ca and cCa assessment should be conducted upon patient initial presentation and disturbances in Ca and cCa levels should be monitored throughout the course of the disease in order to perform timely and appropriate corrective actions. Further research based on larger prospective cohort studies is necessary to confirm the findings presented in this study and to establish the clinical significance of our findings.

Authors' Contribution

Study Conception: İE, HÖ, MB, İKU, AÇ, RG; Study Design: İE, RG, AK, HU, ÖT; Supervision: AK, HU, ÖT; Funding: İE, MB, AÇ, RG; Materials: İE, MB, AÇ, RG; Data Collection and/or Processing: İE, MB, AÇ, RG; Statistical Analysis and/or Data Interpretation: İE, RG, AK, HU, ÖT; Literature Review: İE, HO, MB, İKU, AÇ, RG; Manuscript Preparation: İE, RG, AK, HU, ÖT and Critical Review: İE, AK, HU, ÖT.

Conflict of interest

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Cardiovascular diseases and risk factors in kidney transplant candidates

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ABSTRACT

Objectives: Cardiovascular diseases and risk factors are associated with adverse cardiac events following kidney transplant. Therefore, pre-transplant evaluation of cardiovascular diseases and risk factors is important for determining the cardiac risk. The objective of this study is to determine the prevalence of cardiovascular diseases and risk factors in kidney transplant candidates.

Methods: This is a single-center and observational study which includes 174 end-stage renal disease patients (male: 55.2% and mean age: 49 ± 13 years) who underwent kidney transplant. Preoperative clinical characteristics, cardiovascular diseases and risk factors of the patients were examined retrospectively.

Results: The study population had multiple cardiovascular risk factors such as hypertension (66.7%), diabetes (28.2%), dyslipidemia (29.9%) and smoking (30.5%) in the evaluation conducted prior to kidney transplant. The most common cardiovascular diseases were detected as congestive heart failure (10.9%) and coronary artery disease (8.6%). The rate of the patients who underwent myocardial revascularization (percutaneous coronary intervention or coronary artery bypass grafting) before kidney transplant was 6.9%. The patients' mean left ventricular mass index was 114 ± 32 g/m2, and 55.5% of the study population had left ventricular hypertrophy.

Conclusions: In our study, the prevalence of cardiovascular diseases and risk factors in the patients who underwent kidney transplant was found to be high.

Keywords: cardiovascular disease, kidney transplantation, risk factor

Cardiovascular diseases and cardiovascular risk factors such as hypertension, diabetes mellitus, dyslipidemia, smoking, left ventricular hypertrophy are commonly seen in patients with end-stage renal disease (ESRD) [1, 2]. The prevalence of coronary artery disease in ESRD patients who received chronic dialysis was found to be 40%, while the prevalence of left ventricular hypertrophy was detected as 75%, and it is known that the risk of cardiovascular morbidity and mortality has considerably increased in these patients [3]. Kidney transplant is the most appropriate treatment strategy for patients with ESRD, and it is associated with lower cardiovascular mortality rates compared to chronic dialysis therapy [4]. Moreover, cardiovascular diseases are more commonly seen in patients who undergo kidney transplant compared to the general population and continue to be the leading cause of post-transplant mortality and morbidity [5].

Current guidelines recommend a detailed pre-operative cardiovascular evaluation and assessment of the patient's individual risk status in ESRD patients who will undergo kidney transplant [6, 7]. In these patients, evaluation, treatment and control of cardiovascular diseases and risk factors are very important in

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terms of preventing undesired post-transplant cardiac events [8]. However, in Turkish population, information regarding the frequency of cardiovascular diseases and risk factors in ESRD patients who will undergo kidney transplant is limited.

The objective of this study is to determine the frequency of cardiovascular diseases and cardiovascular risk factors in ESRD patients who will undergo kidney transplant.

METHODS

The present study was conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by Baskent University Institutional Review Board (Project no: 94603339-604.01.02/9412 - KA20/100) on 10/03/2020.

Study Population

One hundred seventy-four ESRD patients, who underwent kidney transplantation at Başkent University Istanbul Health Practice and Research Center Hospital between January 2011 and January 2017, were included in this study. The patients with ESRD under 18 years of age and the patients whose reliable medical archive records could not be reached were not included in the study.

The patients' demographic characteristics, etiologies of chronic renal failure, type and duration of the dialysis treatment applied, cardiovascular diseases and cardiovascular risk factors, medical treatments they received and laboratory analyses obtained during the cardiological examination performed before the transplant, electrocardiographic and echocardiographic characteristics were retrospectively obtained from hospital's medical records.

Definitions

In the pre-transplant evaluation, hypertension was defined as the detection of systolic blood pressure as ≥ 140 mmHg and/or diastolic blood pressure as ≥ 90 mmHg in the consecutive two blood pressure measurements. The patients, who were previously diagnosed with hypertension and/or used antihypertensive drugs, were accepted as hypertensive patients regardless of the value of the measured blood pressure [9]. Diabetes mellitus was accepted as the detection of fasting plasma glucose as ≥ 126 mg/dL or HbA1c value as ≥ 6.5% in the blood glucose measurement. The patients with a previous diagnosis of diabetes mellitus and/or using antidiabetic medication or insulin therapy were accepted as diabetic patients regardless of their blood glucose and HbA1c values [10]. Hyperlipidemia was accepted as the detection of low-density lipoprotein value as 130 mg/dL or total cholesterol value as ≥ 200 mg/dL or triglyceride value as ≥150 mg/dL in blood lipids measurement. The patients, who were previously diagnosed with hyperlipidemia and/or received lipid-lowering medication were accepted as hyperlipidemic patients regardless of their measured lipid values [11]. Occlusive coronary artery disease was defined as the detection of ≥ 50% stenosis in the left main coronary artery and/or ≥ 70% stenosis in the left anterior descending and/or circumflex and/or right coronary arteries in coronary angiography and/or the

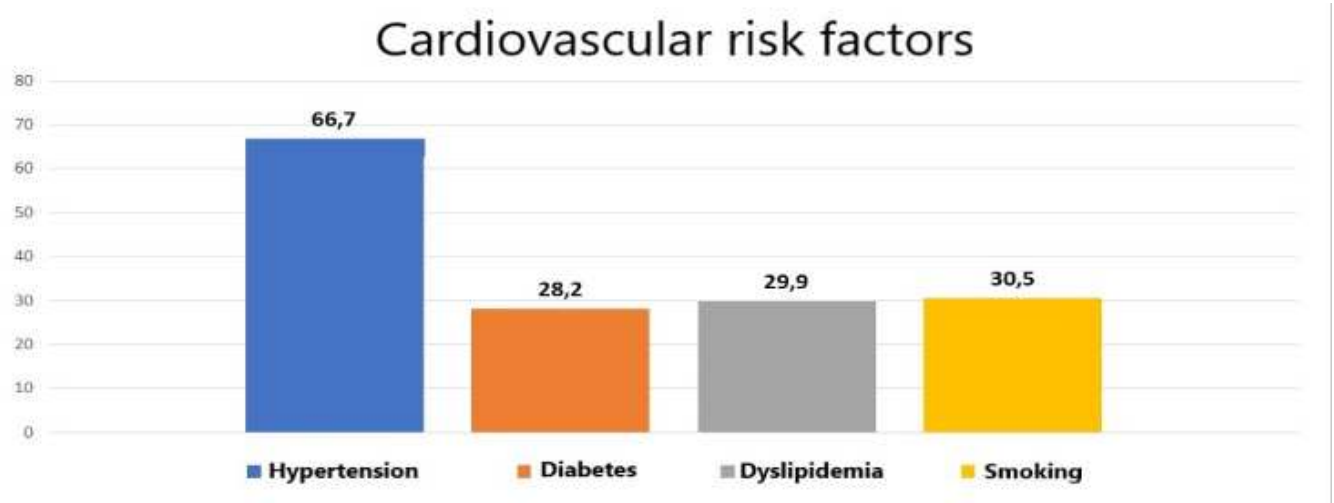

Fig. 1. The figure shows the prevalence distribution of cardiovascular risk factors in the study population.

application of coronary revascularization [12]. Congestive heart failure was defined as the detection of left ventricular ejection fraction as < 50% together with heart failure symptoms and findings or the detection of natriuretic peptide elevation and/or left ventricular hypertrophy and/or left atrial dilatation and/or left ventricular diastolic dysfunction in the patients with heart failure symptoms and findings and left ventricular ejection fraction as ≥ 50% [13]. Left ventricular mass index was calculated by using the Devereux formula in the light of the echocardiographic data obtained [14]. Left ventricular hypertrophy was defined as the detection of left ventricular mass index as > 95 g/m^2 in female patients and > 115 g/m2 in male patients.

Statistical Analysis

Statistical analyses of the study were performed by using the SPSS software package (SPSS, Inc., Chicago, IL, USA) version 22.0. Continuous variables were expressed as mean ± standard deviation (mean ± SD) or median and range, while categorical variables were presented as frequency and percentage (%).

RESULTS

The mean age of 174 patients included in the study was 49.2 ± 13.0 years, while 96 patients (55.2%) were male. The demographic and clinical characteristics of the patients are shown in Table 1. One hundred forty-five (83.3%) ESRD patients were receiving dialysis treatment. The median dialysis treatment duration before kidney transplant was 24 months. Ten patients (5.7%) had previously undergone kidney transplant. When it was evaluated in terms of ESRD etiology, the most common causes were hypertensive nephropathy (32.2%), diabetic nephropathy (18.4%), glomerulonephritis (17.8%) and polycystic kidney disease (9.2%). The etiology of renal failure was not detected in 18.4% of the patients.

The most common cardiovascular risk factors were hypertension (66.7%), smoking (30.5%), hyperlipidemia (29.9%) and diabetes mellitus (28.2%) (Fig. 1). The most common accompanying cardiovascular diseases in the patients with ESRD were congestive heart failure (10.9%) and obstructive coronary artery disease (8.6%). Only 2 patients had a history of atrial fibrillation, and 1 patient had a history of cerebrovascular disease (Fig. 2). The most common non-cardiac comorbidities were anemia (76.4%), hyperthyroidism or hypothyroidism (9.8%), major depression (9.8%) and chronic obstructive pulmonary disease (3.4%) (Table 1).

Before kidney transplant, 47.7% of the patients were using dihydropyridine group calcium channel blocker, 33.3% beta adrenergic receptor blocker, 26.4% antiaggregant treatment, 14.9% diuretic treatment, 14.4% renin angiotensin system blocker, 14.4% alpha adrenergic receptor blocker and 12.6% statin treatment (Table 2).

In the pre-transplant evaluation of the ESRD patients included in the study, the median serum creatinine value was detected as 7.0 mg/dL (range: 2.1-16.0

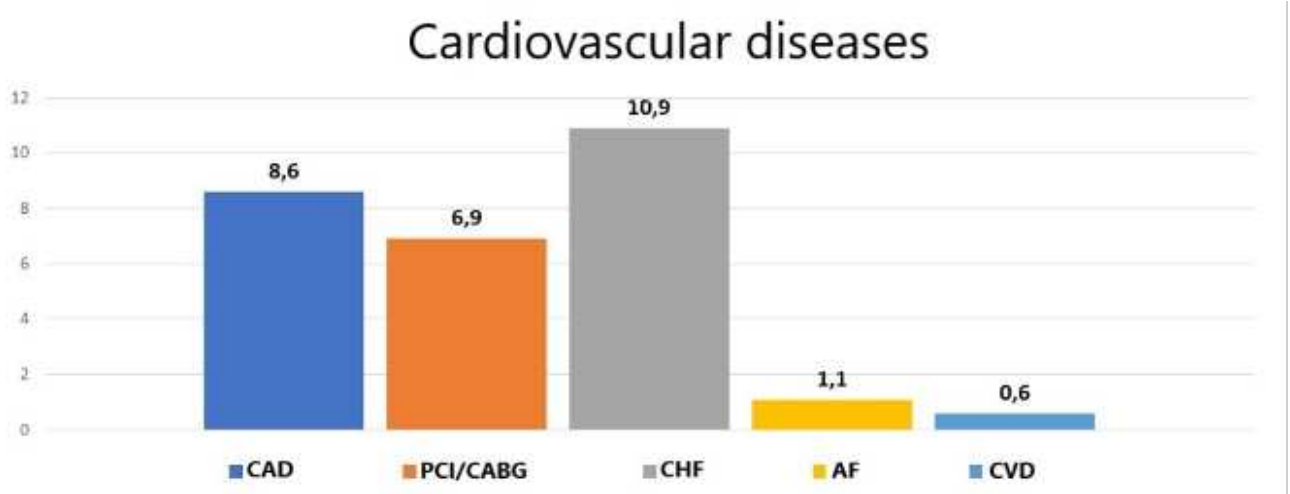

Fig. 2. The figure shows the prevalence distribution of cardiovascular diseases in the study population. (CAD = coronary artery disease, PCI = percutaneous coronary intervention, CABG = coronary artery bypass grafting, CHF = congestive heart failure, AF = atrial fibrillation, CVD = cerebrovascular disease).

Table 1. Clinical characteristics of kidney transplant candidates

Variables	n = 174
Age, years	49.2 ± 13.0
Sex, male	96 (55.2)
Dialysis treatment before kidney transplant	145 (83.3)
Dialysis type	
Hemodialysis	126 (72.4)
Peritoneal dialysis	19 (10.9)
Dialysis treatment duration before kidney transplant (months)	24 (1-240)
History of kidney transplantation	10 (5.7)
Donor type	
Living donor	147 (84.5)
Deceased donor	27 (15.5)
End-stage renal disease etiology	
Hypertensive nephropathy	56 (32.2)
Diabetic nephropathy	32 (18.4)
Glomerulonephritis	31 (17.8)
Polycystic kidney disease	16 (9.2)
Connective tissue disease	4 (2.3)
Renovascular disease	3 (1.7)
Unknown	32 (18.4)
Cardiovascular risk factors	
Hypertension	116 (66.7)
Diabetes mellitus	49 (28.2)
Dyslipidemia	52 (29.9)
Smoking	53 (30.5)
Cardiovascular diseases	22 (12.6)
Obstructive coronary artery disease	15 (8.6)
History of myocardial revascularization	12 (6.9)
Heart failure	19 (10.9)
Atrial fibrillation	2 (1.1)
Cerebrovascular disease	1 (0.6)
Other chronic conditions	
Chronic obstructive pulmonary disease	6 (3.4)
Anemia	133 (76.4)
Thyroid disease	17 (9.8)
Depression	17 (9.8)

Data are given as mean ± SD or n (%).

Tablo 2. Medical treatments, laboratory results, electrocardiographic and echocardiographic data of kidney transplant candidates

Variables	n = 174
Medical treatments	
Renin angiotensin system inhibitors	25 (14.4)
Beta – blockers	58 (33.3)
Diuretics	26 (14.9)
Calcium channel blockers	83 (47.7)
Alpha blockers	25 (14.4)
Statins	22 (12.6)
Antiaggregant/anticoagulants	46 (26.4)
Laboratory data	
Serum creatinine (mg/dL)	7.0 (2.1-16.0)
Fasting blood glucose (mg/dL)	102 (76-552)
HbA1c (%)	5.2 (3.5-9.6)
Low-density lipoprotein cholesterol (mg/dL)	111 ± 40
Hematocrit (%)	30.8 ± 5.4
C-reactive protein (mg/dL)	4.0 (0.2-98)
Thyroid stimulating hormone (mU/L)	1.5 (0.006-8.5)
Parathyroid hormone (pg/dL)	264 (17-2071)
Electrocardiographic data (117 patients)	
Sinus rhythm	115/117 (98.3)
Heart rate (bpm)	79 ± 13
Left bundle branch block	2/117 (1.7)
Non-Left Bundle Branch Block	4/117 (3.4)
Echocardiographic data (164 patients)	
Left ventricular ejection fraction (%)	58 ± 4
Left atrial diameter (mm)	38 ± 6
Mitral E velocity (cm/s)	79 ± 24
Mitral A velocity (cm/s)	76 ± 22
E/A ratio	1.1 ± 0.4
Posterior wall thickness (mm)	11.9 ± 1.7
Interventricular septum thickness (mm)	12.1 ± 1.8
Left ventricular hypertrophy	91/164 (55.5)
Left ventricular mass index (g/m2)	114 ± 32
Moderate-to-severe mitral regurgitation	25/164 (15.2)
Moderate-to-severe tricuspid regurgitation	16/164 (9.8)
Systolic pulmonary artery pressure (mm Hg)	40 (25-65)

Data are given as mean ± SD or n (%).

mg/dL), the median fasting glucose 102 mg/dL (range: 76-552) and the median HbA1c value 5.2% (range: 3.5%-9.6%). The mean low-density lipoprotein value of the patient population was 111 ± 40 mg/dL, and the mean hematocrit value was 30.8% ± 5.4% (Table 2).

Prior to the transplant, electrocardiographic evaluation data were obtained in 117 of 174 patients included in the study. It was observed that 98.3% of these patients were had normal sinus rhythm, and their mean heart rate was 79 ± 13 beats/minute. Only 2 patients (1.7%) had left bundle branch block and 4 patients (3.4%) had right bundle branch block or intraventricular conduction disturbance. Transthoracic echocardiographic examination was performed in 164 of 174 patients before kidney transplant. The mean left ventricular ejection fraction of the patient population was 58% ± 4%, while the mean left atrium diameter was 38 ± 6 mm. The mean left ventricular mass index was found as 114 ± 32 g/m2, and the left ventricular hypertrophy was detected in 91 patients (55.5%). Moderate and/or severe mitral insufficiency was observed in 15.2% of the patients who underwent echocardiographic examination, and moderate and/or severe tricuspid insufficiency was observed in 9.8% of the patients. The median systolic pulmonary artery pressure of the patients was found as 40 mmHg (range: 25-65 mmHg) (Table 2).

DISCUSSION

In the present study, the prevalence of accompanying cardiovascular diseases and cardiovascular risk factors as well as medications, laboratory data, electrocardiographic and echocardiographic characteristics of the patients with ESRD, who underwent kidney transplant, were determined.

The age of kidney transplant in patients with ESRD varies between 43-50 years, and 58% -61% of these patients consist of male patients [15-19]. The age of the patients included in our study was found as 49 years in accordance with the literature, and 55% of them were male.

In the patients undergoing kidney transplant, long-term dialysis treatment before transplant and transplant from cadaver were found to be associated with an increased incidence of adverse cardiovascular events in the post-transplant period [20, 21]. Previous studies reveal that the duration of dialysis treatment in the pre-transplant period varies between 18 months and 36 months on average [15, 16, 22, 23]. In our study, the mean dialysis treatment duration before transplant was determined to be 24 months similar to the previous studies. One of the most important differences of our study compared to the literature is the donor-type distribution. Literature data reveal that kidney transplant in patients with ESRD is mostly performed from cadavers rather than living donors. This rate varies between 83% and 92% [15,16]. In our study, differently from the literature, it was found that only 15.5% of the patients underwent kidney transplant from a cadaver, and 84.5% of the patients underwent kidney transplant from a living donor. This situation reveals the inadequacy of organ donation in Turkey and shows that studies that will be conducted countrywide are needed to popularize organ donation.

Our results put forward that the most common cardiovascular risk factor in patients with ESRD who undergo kidney transplant is hypertension. In our study, the prevalence of hypertension was found as 66.7%. While the prevalence of hypertension is between 21% and 26% in the general population, this rate varies between 67% and 86% in patients with ESRD. This is similar to our study data [16, 18, 19, 24].

The prevalence of diabetes mellitus in patients undergoing kidney transplant varies widely from study to study. The study conducted by Fazelzadeh *et al*. evaluated 500 patients who underwent kidney transplant and found the prevalence of diabetes as 7% [18]. On the other hand, in a comprehensive study conducted by Goyal *et al*. [17] where they evaluated 147,431 patients who underwent kidney transplant, the prevalence of diabetes was found to be 24%. The prevalence of diabetes mellitus in our study was 28.2%, and it was higher than other studies where kidney transplant candidates were evaluated [17-19, 24, 25]. According to the assessment results of the European Society of Cardiology 2019 Cardiovascular Diseases Statistics [26], the median prevalence of diabetes mellitus in the general population in the 20-79 age group was 6.8%; this rate was found to be 12.1% in Turkey. Our country is the third country with the highest prevalence of diabetes after Egypt and Lebanon. Similarly, in Turkey, the prevalence of obesity (body mass index ≥30 kg/m2) was reported as ~25% in males and ~39% in females. The high prevalence of diabetes

in our study population may be a result of the increased prevalence of obesity and diabetes in the general population.

The presence of accompanying cardiovascular disease in patients with ESRD is one of the strongest predictors of adverse cardiovascular events in the post-transplant period. According to the results of a study conducted by Aalten *et al*. [15] where 2187 patients, who underwent kidney transplant, were evaluated, the presence of cardiovascular disease before transplant increased the risk of undesired cardiovascular events in the post-transplant period by 76%. This result was confirmed by other observational studies evaluating kidney transplant patients [16, 22]. The study results show that the prevalence of cardiovascular disease varies between 7% and 16% in ESRD patients undergoing kidney transplant [15, 16, 22]. In our study, it was found that 8.6% of the patients had obstructive coronary artery disease, and 10.9% had congestive heart failure, in accordance with the current data.

In this patient group, another important cardiovascular risk factor is smoking. Studies reveal that active smoking or the history of smoking is associated with the development of cardiovascular complications in the post-transplant period [19, 22, 23]. Gonçalves *et al*. [19] reported that the 7-year all-cause mortality risk was higher than 10% in kidney transplant individuals who were smoking actively or who smoked in the past. Similarly, Chuang *et al*. [23] found that pre-transplant smoking increased the risk of developing acute coronary syndrome 3.5 times more in the early period of the first 2 years after the transplantation. In studies involving kidney transplant patients, the smoking rate was quite high and ranged from 25% to 50% [16, 19, 22]. In our country, the prevalence of smoking in the general population aged 15 years and above varies between 40-45% in males and 10-15% in females [26]. Our study shows that approximately one third of the patients who undergo kidney transplant actively smoke or have a history of smoking. In our study, the prevalence of smoking was found to be 30.5% among patients, and this rate almost coincides with the rates in the general population. This situation shows that the efforts and campaigns across the country to provide support for smoking cessation are still insufficient. More widespread and aggressive strategies are needed across the country in order to achieve a reduction in terms of smoking.

The presence of left ventricular hypertrophy is a risk factor for the development of cardiovascular diseases [9]. The presence of left ventricular hypertrophy in the patients with ESRD is associated with the development of ischemic heart disease, congestive heart failure and ventricular arrhythmia [27]. The prevalence of left ventricular hypertrophy in patients undergoing kidney transplant ranges from 45% to 75%. This significant difference revealed in different studies is due to the lack of a standard approach in the diagnostic methods and estimation values used to detect left ventricular hypertrophy. In our study, the prevalence of left ventricular hypertrophy was found as 55.5%.

Limitations

The most important limitations of our study are its retrospective design and the inclusion of the patients from a single center. The relatively limited number of the patients included in the study reduces the statistical power of the study. Retrospective data were based on the documentation of medical history, clinical examination and treatments during the preoperative cardiac examination, and follow-up data after renal transplantation were not obtained. Thus, the rehospitalization and mortality rates of the patients after transplantation are unknown. Because of these limitations, the results of this study should be interpreted carefully.

CONCLUSION

Our study reveals that the prevalence of cardiovascular disease and cardiovascular risk factors is high in ESRD patients undergoing kidney transplant. Therefore, pre-operative cardiovascular risk assessment is important in terms of minimizing the risk of adverse events in the post-transplant period in ESRD patients who are kidney transplant candidates.

Authors' Contribution

Study Conception: UK; Study Design: UK; Supervision: UK; Funding: UK; Materials: UK; Data Collection and/or Processing: UK; Statistical Analysis and/or Data Interpretation: UK; Literature Review: UK; Manuscript Preparation: UK and Critical Review: UK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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The European Research Journal 2021;7(5):533-540 Case Report

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COVID-19 pneumonia with ARDS and secondary haemophagocytic lymphohistiocytosis: a case report

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ABSTRACT

Although most people with COVID-19 have mild to moderate symptoms; some people may experience worsened symptoms, such as pneumonia, acute respiratory distress syndrome (ARDS) and and respiratory failure. Here we reported a case of COVID-19 pneumonia with ARDS and secondary haemophagocytic lymphohistiocytosis (sHLH). A 44-year-old man presented to the emergency department with a history of cough, fever, sore throat, and muscle ache. The patient transferred to Chest Diseases Department, with a diagnosis of COVID-19 pneumonia. Oseltamivir, hydroxychloroquine, azithromycin, low molecular weight heparin (LMWH) and favipravir added to the treatment. Tocilizumab started after endotracheal intubation. In general, we saw improvement in respiratory values and vital signs. Administration of LMWH to the patient may have prevented the development of coagulopathy. In conclusion, using HFO system and using low tidal volume according to ARDS net protocol, using optimal PEEP/FiO2 ratio, paying attention to keeping driver pressures low may be beneficial for the clinical improvement of the disease.

Keywords: COVID-19, respiratory failure, pneumonia, ARDS, tocilizumab

COVID-19 is the infectious disease caused by the most recently discovered coronavirus. This new virus and disease were unknown before the outbreak began in Wuhan, China, in December 2019. COVID-19 is now a pandemic affecting many countries globally. The most common symptoms of COVID-19 are fever, dry cough, and tiredness. Some people become infected but only have very mild symptoms. Most people (about 80%) recover from the disease without needing hospital treatment. Around 1 out of every 5 people who gets COVID-19 becomes seriously ill and develops difficulty breathing [1].

CASE PRESENTATION

A 44-year-old man presented to the emergency department with a history of cough, fever, sore throat, and muscle ache. He had no history of use medications, smoking and alcohol. On physical examination, no abnormalities were found. Clinical examination revealed a temperature of 38°C, a pulse rate of 112 beats per minute, a blood pressure of 120/80 mm Hg. He had rare inspiratory rales in the basal lungs. No feature was detected in other system examinations. Laboratory results were as follows: white blood count count (WBC) of 6.8 L×103/uL (normal 5.2-12.4 L×103/uL), a hemoglobin (Hgb) of 13.8 g/dL (normal: 12-18 g/dL) and a platelet count of 240×103/uL (normal: 130-400×103/uL), C-reactive protein of 19.4 mg/dL (normal: 0.5-0.9 mg/dl), ferritin 518 (normal 12-300 ng/mL), D-dimer of 631µg/L, INR of 0.96 (normal: 0.8 to 1.1) and procalcitonin of < 0.12 ng/ml (normal: 0.00-0.05 ng/ml).

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There was more peripheral density increase in the left lung lower zone on chest radiograph (Fig. 1). There were multiple ground-glass areas in the thorax CT, mostly in both lower lobes and peripheral regions (Fig. 2). The patient transferred to Chest Diseases Department, with a diagnosis of COVID-19 pneumonia. Hydroxychloroquine 2×400 mg (bolus), azithromycin 1×500 (bolus), influenza pneumonia, oseltamivir 2×75 mg and LMWH treatment at prophylactic dose were administered to the patient. The PCR sample taken from the patient twice; although the first was negative, the second test was evaluated as positive. On the 5th day of hospitalization, the general condition deteriorated and the patient's SpO_2 decreased to 83% despite 3 L/minute oxygen inhalation by face mask. The leukocyte, platelet, ferritin, D-dimer, CRP and INR values were increased, and the lymphocyte and hemoglobin values were decreased (Table 1). The patient's control chest x-ray showed an increase in heterogeneous density in both middle and lower zones of the lung. We observed worsening in the first radiograph compared to the other (Fig. 3). The patient was taken to the intensive care unit because of the his current findings, and favipravir 2×1600 mg (bolus) and piperacilin-tazobactam 3×4.5 g and levofloxacin 1×750 mg were added to his current treatment. As the patient's oxygen saturation was low, high flow oxygen therapy (HFO) was started (40 L/ min flow and FIO2 100%). On day 10 of hospitalization the patient was intubated and connected to the mechanical ventilator (Table 2) (Fig. 4) (respiratory rate of 42 breaths per minute, oxygen saturation of 80%, increased leukocyte, D-dimer, ferritin, CRP and procalcitonin values, worsening arterial blood gas values and worsening on chest x-ray).

The patient's syptoms were moderately compatible with ARDS (PEEP / Fi02 = 123). Therefore, MV was applied to the patient with low tidal volume (4-6 ml/kg), low plateau pressure (< 30 cm H2O), low drive pressure (< 15 cm H2O) and optimal PEEP/Fi02 values in accordance with the ARDS net protocol. The patient was started to be followed up in VC-SIMV mode with a dose of 0.02-01 mg/kg/h with midazolam (Table 3). As the patient's clinical and laboratory values were compatible with secured hemophagocytic lymphohistiocytosis (sHLH), tosilizumab (400 mg+400 mg) treatment was started in addition to the current treatment. On the 15th day of hospitalization (on the 5th day of intubation) respiratory values (Table 2) and laboratory values (Table 1) of the patient improved. Regression was observed on the chest X-ray of the patient (Fig. 5). Upon improvement in the general condition of the patient, his sedation was reduced and discontinued. The patient was extubated according

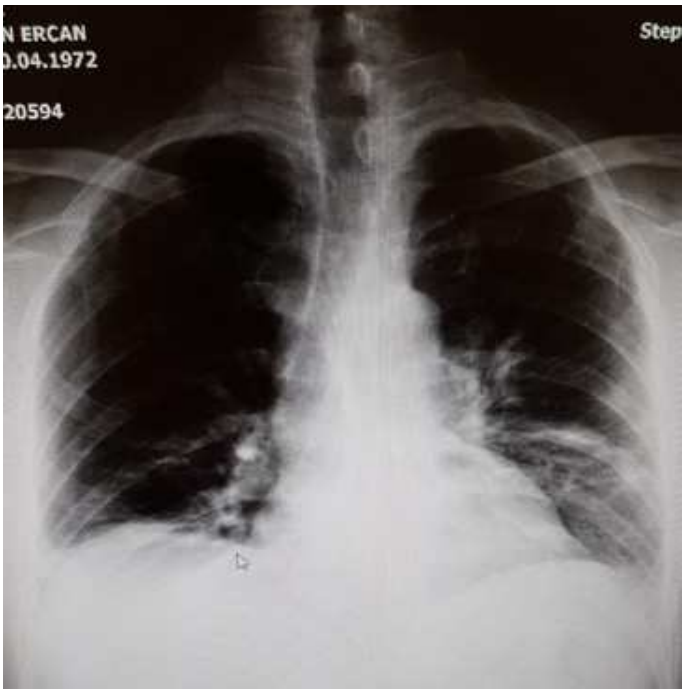

Fig. 1. Hospitalization Chest X-ray

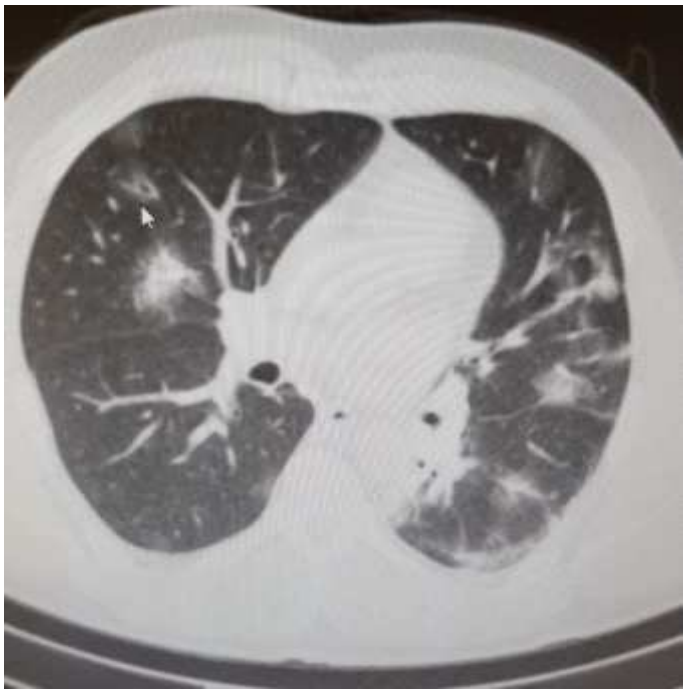

Fig. 2. a-Hospitalization thorax CT.

Table 1. Laboratory results of the patient

Parameters	Day 1 (hospitalization)	Day 5 (HFO)	Day 10 (IMV)	Day 13 (IMV)	Day 17 (O2)	Day 20 (Room)
Leukocyte* ($\times 10^3/\mu L$)	6800	13000	15800	7000	7200	8100
Lymphocytet# ($\times 10^{-3}/\mu L$)	2	1	0.59	0.71	1.86	2.68
Hb# (g/dL)	13.8	11.8	9.6	8,3	10,3	11.2
Platelets	240000	350000	546000	377000	354000	283000
Ferritin*	518	673	710	612	654	733
Fibrinogen# (mg/dL)	417	398	372	358	276	209
D-dimer* (µg/L)	631	1090	6390	2430	1867	443
Troponin (ng/L)	< 10	< 10	< 10	< 10	< 10	< 10
INR*	0.96	1.12	1.7	1,8	1.4	0.99
CRP* (mg/L)	19.4	144	171	25	12	< 3
Procalcitonin* (ng/ml)	< 0.12	0.8	1.3	0.32	< 0.12	< 0.12
ALT (u/L)	26	58	46	82	105	129
AST (u/L)	30	33	44	58	83	55
CK (u/L)	134	128	136	140	142	143
LDH u/L	278	329	341	422	310	258
Triglyceride (mg/dL)	256	260	302	384	414	551

The parameters marked * showed a positive correlation with the progression of the disease, and a positive correlation with the # marked parameter.

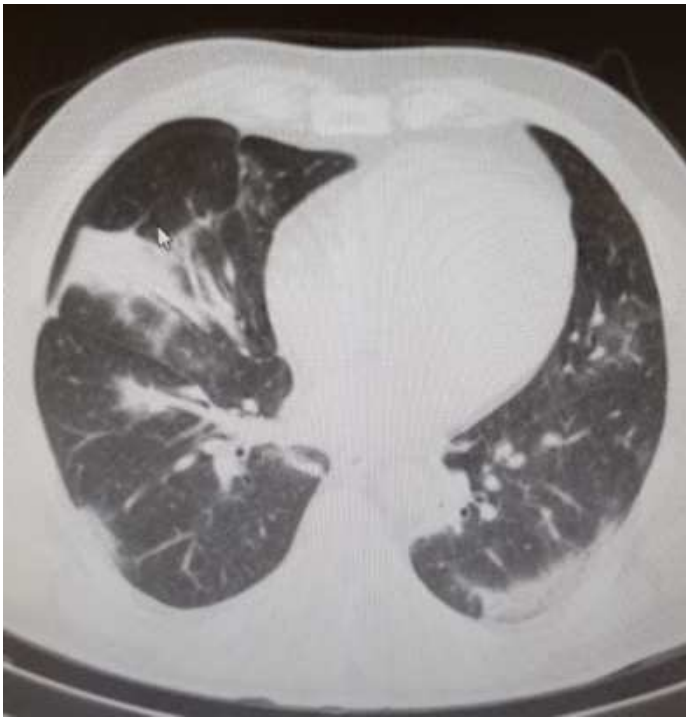

Fig. 2. b-Hospitalization thorax CT.

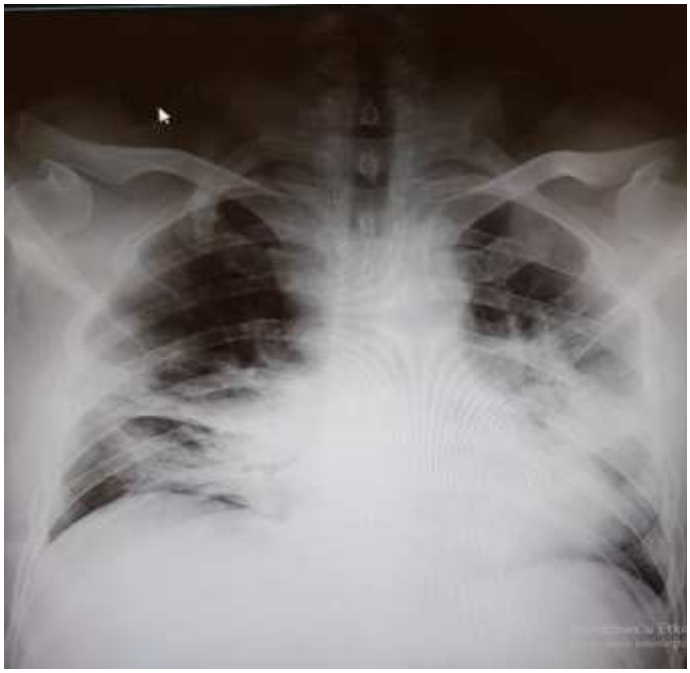

Fig. 3. Intensive care hospitalized chest X-ray (Day 5 of hospitalization).

to the weaning protocol on the 17th day of hospitalization and 7 days after intubation. The patient was transferred to the chest service. Patients' laboratory values (Table 1) and chest x-ray result found normal (Fig. 6). The patient was discharged with a prophylactic dose of LMWH.

DISCUSSION

The vast majority of COVID-19 patients presented with lymphocytopenia (83.2%), whereas 36.2% had thrombocytopenia, and 33.7% showed leukopenia, increased values of CRP (75-93% of cases), LDH (2-92% of cases), ESR (up to 85% of cases) and D-dimer

Table 2. Respiratory values of the patient

Parameters	Day 1 (hospitalization)	Day 5 (HFO)	Day 10 (IMV)	Day 13 (IMV)	Day 17 (O2)	Day 20 (Room)
Respiratory rate (Lt / min)	18	28	42	20	19	16
PH		7.49	7.37	7.48	7.46	
PCO2 (mmHg)		30	60.2	38	39	
PO2 (mmHg)		96	74	63,8	67	
HCO3 (mEq/L)		25	31	29	28	
O2 sat (%)	92	97	80	91	95	97
Lactate (mmol/L)		1.4	2.1	1.2	1.1	
PaO2/FiO2		118	123	116	167	320
FiO2 (%)		100	60	55	40	Room

Table 3. Monitored values in patient's ventilator follow-up

	Day 10 (IMV)	Day 13 (IMV)	Day 17 (O2)
MODE	VC-SIMV	VC-SIMV	VC-SIMV
FiO2	60	55	40
PaO2/FiO2	123	116	167
VT	400	400	400
T isn	1.5	1.5	1.5
PEEP (cmH20)	15	12	5
Support Pressure (cmH20)	20	18	10
Insp, flow (L/min)	45	50	45
MVE (L/min)	12.3	8.9	7.3
VTE (ml)	420	370	372
RR	18	18	18
TOTAL RR	22	20	19
Plateau Pressure (cmH20)	27	24	16
Drive Pressure (cmH20)	12	12	11
Resistance	16.4	10	8.4
Dynamic Compliance (mL/cmH2O)	70	94	103

VT = tidal volume, Ti = inspiration time, PEEP = positive end expiratory pressure, MVE = minute ventilation, VTE = vital capacity RR = number of breaths

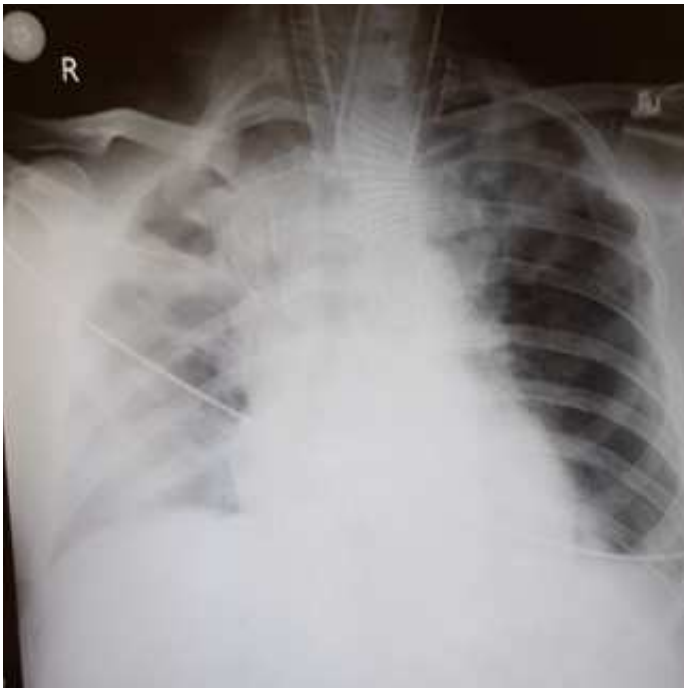

Fig. 5. Chest x-ray (transferred from the intensive care unit to the service-Day 17 of hospitalization).

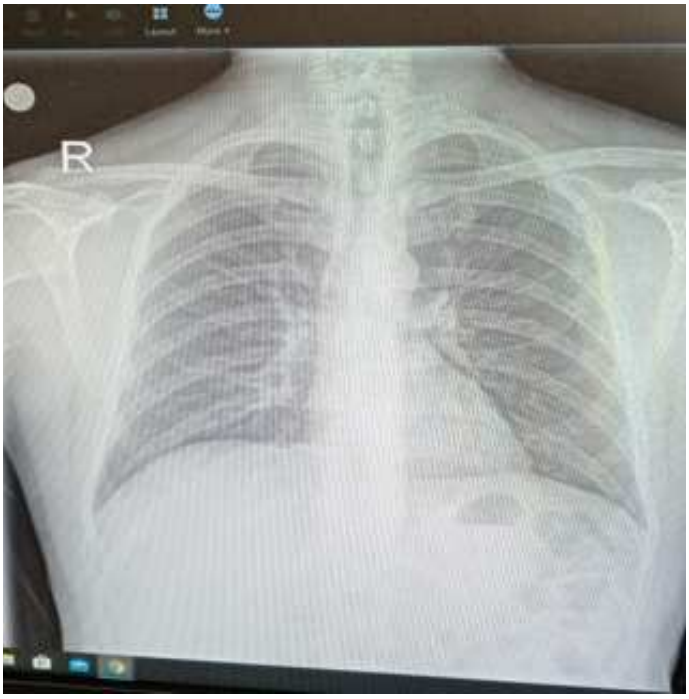

Fig. 6. Chest X-ray, Discharged (Day 20 of hospitalization)

(36-43% of cases), as well as low concentrations of serum albumin (50-98% of cases) and hemoglobin (41-50%) [6-8]. Increased white blood cell count, increased neutrophil count, decreased lymphocyte count, decreased albumin, increased LDH, ALT, AST, bilirubin, creatinine, cardiac troponins, D-dimer, prothrombin time, procalcitonin and CRP values. In our case, leukocyte, CRP, procalcitonin, ferritin, INR, triglyceride, D-Dimer values increased as disease progression increased. The lymphocyte count and hemoglobin values of the patient decreased as the disease progressed (Table 1). In our case, AST, ALT, LDH, CK, fibrinogen, platelet values did not correlate with the patient's progression. In addition, the patient's increased triglyceride values did not reach normal values despite the patient's clinical and radiological regression. During hospitalization, a nasopharyngeal swab was taken 2 times with an interval of 48 hours, and a real-time PCR test was performed, but a negative result was found. The first rapid antibody test on the 5th day of hospitalization was negative. The second rapid antibody test was found positive. It is known that viral load is high in nasopharyngeal secretions during the first week. In a study, 63% of nasal swabs and 32% of pharyngeal swabs were detected [9]. However, in real time PCR tests, many factors such as sample sample quality, collection time of clinical samples, and transfer in unfavorable conditions affect the test results. Rapid antibody testing may be negative when performed at an early stage. Positivity rates are maximum 5-14 days after symptoms begin. However, antibody formation may not be the same in every patient. Real-time PCR is the gold standard in the diagnosis of the disease. However, it may show false negativity in the early stage of the disease [10, 11]. It should be borne in mind that in the early stage of the disease, limited frosted glass can not be seen on the chest radiograph. Sensitivity of chest radiography is reported between 30-60% [12]. In this case, chest x-ray showed an increase in heterogeneous density in the left lung lower zone with peripheral location (Fig. 1). However, this image is not specific for COVID-19 pneumonia. Therefore, our patient received a non-contrasted thorax CT. In the thorax CT, multiple frosted glass opacities were noted in all bilateral lung areas, mostly peripheral and in the lower lobe. In the studies conducted, the sensitivity of the real-time PCR test at the early stage of the disease was 71%, and the sensitivity of Torak CT was 98% [10, 11] Consolidation, cobblestone view, air bronchogram, vascular enlargement, bronchial dilatation, halo sign, inverse halo sign, nodules, air bubble (air-bubble), subpleural and parenchy-

mal bands were seen in cases of COVID-19 pneumonia in the literature [13-16]. Progression of consolidation and expansion to the upper lobes, development of pleural or pericardial fluid, development of lymphadenopathy, bronchiectasis, halo sign, cavitation development, and pneumothorax are poor prognostic factors. In our case, radiological progression was observed from the 5th day of hospitalization and the consolidation extended to both apical regions (Fig. 3 and 4). The majority of people infected with COVID-19 are asymptomatic, 14% of patients are hospitalized and 5% of them need intensive care [1]. Currently, there is no vaccine or antiviral treatment for COVID-19. Current treatments are given to prevent the entry of the virus into the cell, reduce its replication and suppress the increased inflammatory response [17]. We applied hydroxychloroquine treatment to the patient since his hospitalization. Because studies have shown that hydroxychloroquine reduces binding of the virus to angiotensin-converting enzyme 2 (ACE2) receptor and reduces the spread of the virus [18]. In addition, it has been shown that chloroxine is effective in preventing the development of the patient's pneumonia, shortening the disease duration and PCR negation [19]. We started Oseltamivir treatment. Because it was determined that 4.3% of COVID-19 patients can have influenza infection at the same time. However, differentiation of influenza pneumonia and COVID-19 pneumonia is not possible radiologically [20]. We started azithromycin as an initial treatment for our patient. Because it is known that macrolides have anti-inflammatory properties such as down regulation and adhesion molecules inhibition, as well as antibacterial properties of proinflammatory cytokines [21]. Therefore, it is thought to be used in COVID-19 pneumonia [22]. Finally, we started a prophylactic dose of LMWH in our patient. Because in COVID-19 infection, there are studies showing that patients with high D-dimer values and severe pictures can be complicated by thrombosis and that such cases are mortal. In autopsy studies, vasculitis, small vessel occlusion, and thrombotic microangiopathy were observed in these patients [23-25]. LMWH treatment reduces the risk of mortality [26]. As a result, we started treatment with oseltamivir, hydroxychloroquine, azithromycin and LMWH. We added favipravir to the current treatment due to the clinical, laboratory and radiological progress monitoring of the patient. Because favipravir is a purine analogue with RNA-dependent RNA polymerase inhibitor (RBRI) and it can be effective in COVID-19 infection just as it is in SARS and MERS since it contains the SARCoV-2 virus RBRI [27]. However, despite the favipravir treatment given, our patient still showed progression. Chen et al. [28] have been reported that abnormal AST and ALT following treatment with favipiravir. We considered secondary hemophagocytic lymphohistiocytosis (sHLH) due to; worsening of breathing, moderate ARDS, continued radiological progression, decreased lymphocyte and hemoglobin values, increased ferritin, D-dimer, triglyceride level and we started to tosilizumab treatment. In studies conducted in China, secondary hemophagocytic lymphohistiocytosis (sHLH) similar picture was observed in COVID-19 patients [8]. This may be due to the fact that the virus activates CD4 + T lymphocytes after entry into the cell, leading to the formation of T hepler (Th), and the activation of inflammatory cytokines by activating the release of IL-6 from monocytes and macrophages through GM-CSF and other inflammatory cytokines (17). Since our patient had bilateral lung infiltration in the last week and PaO_2/FiO_2 ratio was 123, we accepted it as medium ARDS according to Berlin definition [29]. Our patient had bilateral lung infiltration in the last week and PaO2/FiO2 ratio was 123, and we accepted our patient as middle ARDS according to Berlin definition [29]. We used a volume-controlled mode for our patient. We used the low tidal volume (6 ml/kg), optimal PEEP/FiO2 ratio according to the ARDS network table. We tried to comply with the targets of plateau pressure (PPLAT) < 30 cmH20, pH > 7.30, pO_2 > 60 mmHg and spO_2 > 90. Studies show that compliance and shunt fraction may differ from ARDS in patients who develop and intubate with COVID-19 pneumonia. Thorax CT findings of different severity were observed in patients with the same PaO_2/FiO_2 ratio. Symptom onset and radiological involvement of patients may not be compatible with ARDS in all cases. Therefore, it may be beneficial to continue patients with HFO as much as possible without intubation. Gattinoni et al. [30, 31] (Explained hypoxia in 3 different mechanisms in patients with COVID-19 pneumonia.) These are dysregulation in pulmonary perfusion, microtrombus in lung parenchyma, non-cardiogenic pulmonary edema. For this reason, they divided the patients into two groups, H and L feno-

type. The main problem in the L phenotype is perfusion dysregulation and microthrombus. Therefore, patients with this phenotype do not respond to high PEEP, recruitment therapy and pron position. In patients with H phenotype, elastance was high and compliance was low. Therefore, high PEEP can respond to recruitment therapy and pron position. Patients can switch from the L phenotype to the H phenotype [30, 31]. Considering the air resistance and dynamic compliance values in our case, it fits the L phenotype. Therefore, high PEEP, recruitment therapy, prone position and neuromuscular blockade were not required.

CONCLUSION

Approximately 14% of confirmed cases developed severe disease, while the grand fatality rate was 4.2%. No pharmaceutical products have yet been shown to be safe and effective for the treatment of COVID-19. Current treatments are provided to prevent the entry of the virus into the cell, reduce replication and suppress the increased inflammatory response. We gave our patient primarily oseltamivir, hydroxychloroquine, azithromycin, LMWH treatment. However, we added favipravir upon progression. Due to the suspicion of cytokine storm, we started early treatment of tocilizumab. In general condition, respiratory values and vital signs of our patient improved. We observed radiological regression in a short time. Administration of LMWH to the patient may have prevented the development of coagulopathy. Using HFO system and not rushing for intubation, using low tidal volume according to ARDSnet protocol, using optimal PEEP/FiO2 ratio, paying attention to keeping driver pressures low may be beneficial for the clinical improvement of the disease.

Authors' Contribution

Study Conception: HÖ, MT, EÜ; Study Design: HÖ, MT, EÜ; Supervision: HÖ, MT, EÜ; Funding: HÖ, MT, EÜ; Materials: HÖ, MT, EÜ; Data Collection and/or Processing: HÖ, MT, EÜ; Statistical Analysis and/or Data Interpretation: HÖ, MT, EÜ; Literature Review: HÖ, MT, EÜ, NAİ; Manuscript Preparation: HÖ, MT, EÜ, NAİ and Critical Review: HÖ, MT, EÜ, NAİ.

Informed consent

Written informed consent was obtained from the patient for publication of this case and any accompanying images.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Ethical approval

This study was approved by the Ethics Committee of Erzincan University.

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