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# Relationship Between Electrocardiography and Electrolytes Before and After Dialysis in Hemodialysis Patients

## Hemodiyaliz Hastalarında Diyaliz Öncesi ve Sonrası Elektrokardiyografi ile Elektrolitler Arası İlişki

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## Abstract

**Aim**: Cardiovascular events are the most important cause of mortality in hemodialysis patients. Rapid volume changes and electrolyte shifts during dialysis lead to arrhythmias. The study aims to determine whether there is a relationship between before and after hemodialysis electrocardiographic (ECG) whole wave and interval changes and electrolyte levels in hemodialysis patients.

**Material and Method**: A total of 112 patients undergoing maintenance hemodialysis treatment three times a week for four hours for more than six months were included in the study. Before and after hemodialysis, 12-lead ECG, weight, arterial blood pressure, before hemodialysis hemogram and biochemistry, and demographic data were recorded. Waves and intervals were calculated from the 12-lead ECG taken before and immediately after dialysis, and the relationship with serum electrolyte levels was evaluated. P<.05 was considered significant.

**Results**: Among the patients, 51.8% were female, and the mean age was 49.83(±18.69) years. Post-dialysis RR interval showed a significant negative correlation with phosphorus (p=.007) and uric acid (p=.013). A moderate negative correlation was found between the pre-dialysis QTc interval and uric acid (p=.008), and between the post-dialysis QTc interval and sodium (p=.016). Linear regression analysis revealed that phosphorus (p=.007) and uric acid (p=.013) significantly affected the post-dialysis RR interval, uric acid (p=.008) was significant on pre-dialysis QTc interval. Again sodium (p=.016) and calcium (p=.027) were significant for the post-dialysis QTc interval.

**Conclusion**: The negative correlation between post-dialysis RR interval and phosphorus and uric acid, between pre-dialysis QTc and uric acid, and between post-dialysis QTc and sodium makes ECG interpretation and arrhythmia risk assessment difficult as a result of volume and electrolyte shifts.

**Keywords**: Hemodialysis, electrocardiography, electrolytes

## Öz

**Amaç**: Hemodiyaliz hastalarında kardiyovasküler olaylar en önemli mortalite nedenidir. Diyaliz esnasındaki hızlı volüm değişiklikleri ve elektrolit şiftleri aritmilere önderlik eder. Bu çalışma hemodiyaliz hastalarında hemodiyaliz öncesi ve sonrası elektrokardiyografik (ECG) tüm dalga ve interval değişiklikleri ile elektrolit düzeyleri arasında ilişki olup olmadığının belirlenmesini amaçlamaktadır.

**Gereç ve Yöntem**: Çalışmaya altı aydan uzun süre, haftada üç gün dört saat süreyle hemodiyaliz tedavisi gören 112 hasta dahil edildi. Hemodiyaliz öncesi ve sonrası 12-derivasyonlu EKG, kilo, tansiyon arteriyel, hemodiyaliz öncesi hemogram ve biyokimya ile demografik verileri kaydedildi. Diyaliz öncesi ve hemen sonrası çekilen 12-derivasyonlu EKG'den dalga ve intervalleri hesaplanarak serum elektrolit düzeyleri ile arasındaki ilişki değerlendirildi. P<.05 anlamlı kabul edildi.

**Bulgular**: Hastaların %51.8 kadın, ortalama yaş 49.83(±18.69) idi. Diyaliz sonrası RR intervali, fosfor (p=.007) ve ürik asit (p=.013) ile anlamlı negatif korelasyon gösterdi. Diyaliz öncesi QTc intervali ile ürik asit (p=.008) arasında, diyaliz sonrası QTc intervali ile sodyum arasında negatif yönde (p=.016) orta derecede korelasyon bulundu. Lineer regresyon analizinde, fosfor (p=.007) ve ürik asitin (p=.013) diyaliz sonrası RR intervalini, ürik asitin (p=.008) diyaliz öncesi QTc intervalini anlamlı düzeyde etkilediği görüldü. Yine diyaliz sonrası QTc intervali için sodyum (p=.016) ve kalsiyum (p=.027) etkileri anlamlıydı.

**Sonuç**: Diyaliz sonrası RR intervali ile fosfor ve ürik asit arasında negatif yönde, öncesi QTc ile ürik asit arasında ve sonrası QTc ile sodyum arasında negatif yönde bulunan korelasyon, volüm ve elektrolit şiftleri sonucu EKG yorumunu ve aritmi risk değerlendirmesini zorlaştırmaktadır.

**Anahtar Kelimeler**: Hemodiyaliz, elektrokardiyografi, elektrolitler

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## INTRODUCTION

Cardiovascular problems are the most important cause of morbidity and mortality in hemodialysis patients.[1,2] Decreased renal function is associated with an increased incidence of arrhythmia and coronary artery disease.[3] The American renal data system reports that arrhythmia and cardiac arrest are the leading causes of death and account for 40% of deaths in hemodialysis. This rate is 50% in the first three months of starting hemodialysis.

Mortality is expected to be higher given the increasing prevalence of left ventricular hypertrophy and arteriosclerotic disease on hemodialysis.[3] KDIGO practice guidelines recommend ECG at the beginning and follow-up of renal replacement therapy.[4]

Unlike the general population, the main cause of acute cardiac death in dialysis patients is not coronary artery disease, and arrhythmias such as bradycardia, ventricular arrhythmia, and asystole have drawn attention.[5] Ventricular arrhythmias are expected to occur because chronic uremia causes diffuse myocardial fibrosis, coronary calcification, endothelial and autonomic dysfunction, and left ventricular hypertrophy.[6]

The fact that electrocardiography, which is used as a diagnostic tool in cardiovascular diseases, may be affected by volume and electrolyte changes makes ECG interpretation difficult. Metabolic and structural changes in hemodialysis patients are factors in cardiac events.[5]

The P wave, indicating atrial repolarization, is followed by the QRS wave, indicating ventricular depolarization. The QT interval is an indicator of ventricular depolarization and repolarization. Dynamic and variable QT repolarization is especially affected by potassium, magnesium, calcium, and acidosis. Prolonged QT has been linked to acute cardiac deaths and ventricular arrhythmias, with over 480 milliseconds associated with death.[6] Again, longer duration of stay in hemodialysis treatment was associated with QT prolongation.[5] QTc is obtained by correcting the heart rate effect. This study aimed to evaluate the relationship between ECG waves and intervals and electrolytes levels in hemodialysis patients to better understand the potential for arrhythmia risk.

## MATERIAL AND METHOD

The study included 112 patients undergoing hemodialysis three times a week for four hours for more than six months. Electrocardiography (ECG) (MAC 2000 device), weight, systolic and diastolic blood pressure measurements were recorded before and after hemodialysis at the beginning of the week before and after two days of dialysis. White cell count, hemoglobin, C-reactive protein (CRP), sodium, potassium, calcium, phosphorus, magnesium, albumin, intact parathormone (iPTH), thyroid stimulating hormone (TSH), and bicarbonate levels were also recorded before dialysis. Before hemodialysis, electrolytes were measured with the Siemens ADVIA Centour XPT immunoassay system after transferring the blood into a plain tube to the laboratory at room temperature. An automatic 12-lead ECG was taken before dialysis, after resting in the supine position for five minutes before starting the weekly session and before vascular access was made for dialysis. Post-dialysis ECG was obtained with the same device five minutes after leaving the dialysis machine, without changing the supine position. P wave duration, PR interval, RR interval, QRS duration, QT interval, and QTc interval were calculated from the automatic ECG device. All patients were receiving hemodialysis with standard dialysate containing 2.0 mEq/L potassium, 1.25 mEq/L calcium, and 1 mEq/L magnesium.

Exclusion criteria consisted of patients on hemodialysis for less than four hours, patients on hemodialysis for less than six months, patients with hyperkalemia above 6.5 mEq/L, and patients with signs of acute coronary syndrome, significant rhythm disturbance, pacemaker, and malignancy. Since it is known that the risk is high in patients who have just started hemodialysis, patients who completed six months were included in the study. Acute coronary syndrome, known arrhythmia and paced patients were not included in the study because ECG waves and intervals would be affected. Hemodialysis patients are resistant to potassium levels above normal ranges and were not included in the study due to the expected effects on ECG waves and intervals above 6.5 mEq/L.

This study was conducted with the decision of Mardin Artuklu University Non-Interventional Clinical Research Ethics Committee dated 03.05.2023 and numbered 2023/5-10. Informed consent was obtained from all subjects involved in the study.

### Statistical Analysis

Analyses were conducted using UN Statistical Package for Windows 22.0 version (IBM SPSS Statistic, Armonk, NY, IBM Corp.2013). Categorical variable gender was presented as frequency (n) and percentage (%). The conformity of continuous variables to normal distribution was tested with Skewness and Kurtosis and expressed as arithmetic mean, standard deviation values. Values that do not show normal distribution are presented as median and interquartile range. Paired sample t test was applied to P, RR, QT, QTc, systolic, diastolic and weight variables that showed normal distribution before and after dialysis. Two related sample test (Wilcoxon) was applied for PR and QRS that did not show normal distribution. Pearson correlation was applied for the relationship between ECG waves and intervals of normally distributed sodium, potassium, calcium, phosphorus, magnesium electrolytes and uric acid. Correlated parameters were evaluated by univariate and multivariate regression analysis. $p<.05$ was accepted as a significance level.

## RESULTS

The mean age was 49.83 (±18.69) years, and 51.8% of the patients were female (**Table 1**). There was no significant correlation between ECG waves and intervals and CRP, TSH, iPTH, and bicarbonate measured in blood gas, which may have clinical effects. Again, there was no relationship between albumin and electrolytes.

**Table 1. Demographic characteristic of the Group and Laboratory Results**

| | |
|---|---|
| Age | 49.83±18.69 |
| Male/Female | 51.8% / 48.2% |
| Dialysis time (year) | 4 (4.75) |
| KT/V | 1.6 (.45) |
| WBC ($10^3$/uL) | 7.50 ±2.32 |
| Hemoglobin (g/dL) | 10.84 (±1.67) |
| CRP (mg/dL) | 7.9 (15.58) |
| Sodium (mEq/L) | 137.59 (±3.74) |
| Potassium (mEq/L) | 5.09 (±.82) |
| Calcium (mg/dL) | 8.47 (±.95) |
| Phosphorus (mg/dL) | 4.62 (±1.54) |
| Magnesium (mg/dL) | 2.26 (±.56) |
| Uric Acid (mg/dL) | 6.44 (±1.46) |
| Albumin (g/dL) | 3.75 (±.42) |
| PTH (IU/mL) | 437.2 (532.8) |
| TSH (IU/mL) | 1.86 (2.16) |
| Bicarbonate (mmol/L) | 22 (3.78) |
| Before weight (kg) | 65.54 (±16.37) |
| After weight (kg) | 63.09 (±15.95) |

Mean (±sd), median (IQR)

### ECG wave and interval relationship before and after hemodialysis

There was a significant difference in P wave duration (p=.000), RR interval was significantly shorter after dialysis (p=.000), and there was no significant difference in PR interval (p=.151). QT interval showed a significant shortening after dialysis (p=.000). There was a statistically significant difference in QTc interval (p=.000). There were significant differences in weight (p=.000), systolic blood pressure (p=.000), and diastolic blood pressure (p=.000) before and after dialysis (**Table 2**).

### Relationship between ECG waves and intervals and electrolytes and hemodialysis patient follow-ups

There was no correlation between pre- and post-dialysis P, pre- and post-dialysis PR, pre- and post-dialysis RR, pre- and post-dialysis QRS, and pre-and post-dialysis QT waves, and normally distributed and non-normally distributed laboratory parameters. Post-dialysis RR interval was negatively correlated with phosphorus (p=.007) and moderately correlated with uric acid (p=.013).

**Table 2. Comparison of electrocardiographic parameters before and after hemodialysis**

| Parameters | Total | t, z | p |
|---|---|---|---|
| Before P<br>After P | 108 (±18.7)<br>108.1 (±16.7) | -.61 | .000 |
| Before PR<br>After PR | 157 (33.5)<br>152 (28) | -1.43 | .151 |
| Before RR<br>After RR | 717.7 (±99.13)<br>691.2 (±95.11) | -3.63 | .000 |
| Before QRS<br>After QRS | 89.7 (±14.5)<br>88 (14) | -.114 | .909 |
| Before QT<br>After QT | 394 (±31.8)<br>391 (±43.8) | -2.70 | .000 |
| Before QTc<br>After QTc | 443.9 (±31.78)<br>443.2 (±30.57) | -.075 | .000 |
| Before Systolic<br>After Systolic | 143.2 (±26.8)<br>121.1 (±25.5) | -7.96 | .000 |
| Before Diastolic<br>After Diastolic | 85.9 (±13.8)<br>73.1 (±13.5) | -7.31 | .000 |
| Before Weight<br>After Weight | 65.4 (±16.3)<br>63.1 (±15.9) | 8.26 | .000 |

Mean (± sd), median (IQR) P, PR, RR, QRS, QT, QTc: millisecond Weight: Kg

Post-QTc correlated negatively with pre-weight (p=.007) and positively with post-weight (p=.006). Pre-QRS was negatively correlated with age at dialysis (p=.007), and pre-QT was negatively correlated with age at dialysis (p=.039). Pre-systolic blood pressure was positively correlated with pre-PR (p=.028) and post-PR (p=.043), and post-systolic blood pressure was positively correlated with pre-PR (p=.004) and post-PR (p=.000).

Pre-dialysis weight was positively correlated with post-dialysis QRS (p=.044) and post-QTc (p=.007), and post-dialysis weight was positively correlated with post-dialysis QRS (p=.020) and post-QTc (p=.006).

Pre-QTc was negatively (p=.008) moderately correlated with uric acid. Post QTc was negatively (p=.016) moderately correlated with sodium. Phosphorus (p=.007) and uric acid (p=.013) with RR, were found significant in univariate linear regression analysis (**Table 3**).

**Table 3. Univariate linear regression analysis**

| Dependent | Independent | R2 | B | F | t | p |
|---|---|---|---|---|---|---|
| After RR | Phosphorus | .065 | -15.77 | 7.685 | -2.772 | .007 |
| | Uric acid | .055 | -15.20 | 6.422 | -2.534 | .013 |
| Before QTc | Uric acid | .062 | -5.36 | 7.213 | -2.686 | .008 |
| After QTc | Sodium | .052 | -2.07 | 6.016 | -2.453 | .016 |

In multivariate regression analysis for the effects of serum electrolyte levels on ECG waves, uric acid had a significant effect on pre-QTc (p=.019) and sodium (p=.013) and calcium (p=.027) on post-QTc (**Table 4**).

**Table 4. Multivariate regression analysis of electrolytes affecting ECG waves**

| After QTc | Standardized Coefficients | t | p | Correlations | | | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|---|
| | Beta | | | Zero-order | Partial | Part | Tolerance | VIF |
| Sodium | -.240 | -2.516 | .013 | -.228 | -.237 | -.228 | .906 | 1.104 |
| Calcium | -.205 | -2.239 | .027 | -.175 | -.212 | -.203 | .983 | 1.017 |
| Phosphorus | .029 | .300 | .765 | -.075 | .029 | .027 | .862 | 1.160 |
| Magnesium | .116 | 1.244 | .216 | .035 | .120 | .113 | .942 | 1.061 |
| Uric Acid | -.181 | -1.861 | .065 | -.201 | -.178 | -.169 | .865 | 1.156 |
| Before QTc Uric Acid | -.236 | -2.388 | .019 | -.248 | -.226 | -.220 | .865 | 1.156 |

## DISCUSSION

Cardiac events, which are the most important cause of mortality and morbidity in hemodialysis patients, are most frequent in the last 24 hours of a two-day interval without dialysis and the twelve hours after dialysis at the beginning of the week. In addition to the increased incidence of coronary artery disease, ventricular arrhythmias, asystole, and bradycardia have been noted. Uremic environment, volume overload, and electrolyte shifts may cause dangerous arrhythmias during and immediately after dialysis.[5] Due to volume and electrolyte shifts, more arrhythmias are expected during hemodialysis and in the hours following its completion.[3]

Publications comparing all electrolytes with ECG waves are scarce in the literature. In this study in which we examined the relationship between ECG waves and intervals and electrolytes, no significant relationship was found between pre- and post-dialysis PR intervals and pre- and post-dialysis QRS intervals and electrolytes, and there was no significant difference in PR intervals and QRS intervals before and after dialysis. Marano et al. reported that P wave prolongation was associated with interatrial block and atrial fibrillation, and in addition, electrolyte changes during dialysis, especially potassium decrease, further slowed down interatrial conduction.[7] In this study, no relation was found between potassium and P wave and PR interval. The fact that patients with potassium levels above 6.5 mEq/L were not included in the study may not have shown the rapid change effect.

Nishi et al. pointed out that the incidence of atrial fibrillation increased in hemodialysis patients.[8] Yamaguchi et al. reported that QRS amplitude decreased with volume load, PR prolongation, QRS interval, and QT shortening occurred with hyperkalemia, QT prolongation could be masked by hyperkalemia, and the effects of hyperphosphatemia on ECG were not reported. They interpreted that electrolytes were risk factors for noncardiac cardiovascular disease and that low magnesium was the most effective factor.[9] They reported that prolonged QT due to hypocalcemia may not be an indicator of cardiac tissue damage but may be an effect of transient electrolyte abnormalities.[9] In this study, post-RR was negatively correlated with phosphorus and uric acid. Tsampasian et al. stated that high ATP turnover for normal functioning of the heart depends on efficient energy substrate utilization and that heart diseases can be evaluated noninvasively with phosphorus magnetic resonance.[10] The relationship between RR and phosphorus was found to be compatible with the effects of phosphorus in the metabolic process. Jebali et al. found significant changes in heart rate, R wave, T wave, and T wave/R wave (T/R) before and after dialysis only between QTc and serum potassium in multiple regression. They reported that the effects of hyperkalemia were less pronounced in hemodialysis patients.[11] Our potassium data were consistent with this view. Poulikakos et al. also reported that the increase in P wave duration in hemodialysis was inversely associated with potassium and magnesium and positively associated with calcium, and the increase in QRS duration was associated with a decrease in potassium.[12] In this study, no relation was found between potassium, magnesium and ECG waves. Only sodium and calcium were found to be effective in post-dialysis QTc. The reason may be that only preliminary magnesium, sodium and calcium levels were measured in the study, and serial follow-up was not performed.

The relationship between QTc interval and arrhythmias in hemodialysis patients has been shown in many studies. In the present study, a negative correlation was found between post-dialysis QTc and sodium, calcium. Poulikakos et al. reported that low calcium and potassium concentrations in dialysate prolonged QTc.[12] Covic et al. reported that hemodialysis prolongs QTc with rapid electrolyte changes, especially in calcium, and is not significant in the absence of cardiac disease.[13] In this study, the negative effect of calcium on post-dialysis QTc was consistent with the results of this study.

Octavia et al. reported that QT dispersion before and after dialysis was significant but not correlated with clinical factors.[14] Korkmaz et al. also found that hemodialysis did not cause a change in the QRS axis in their study, which suggested that rapid volume and electrolyte concentration changes in hemodialysis would cause ECG changes and arrhythmias.[15] The absence of QT dispersion and QRS prolongation in this study was consistent with the above study.

Watt et al. found that long QTc intervals were associated with prolonged post-dialysis recovery independent of serum electrolytes.[16] They stated that efforts to shorten QTc would contribute positively to the quality of life in hemodialysis patients. In this study, post-dialysis QTc was negatively correlated with sodium. Astan et al. interpreted QRS and QTc prolongation after hemodialysis as a noninvasive marker for ventricular arrhythmias.[17] Matsumoto et al. reported that the

QTc interval was prolonged as the duration of life on dialysis increased.[5] The negative correlation between QTc and sodium after hemodialysis may help shorten QTc with the increase in serum sodium as a result of dialysis output volume withdrawal. Post-dialysis QTc correlation with calcium may constitute a risk for arrhythmias. This problem can be prevented by standardizing the dialysate calcium level.

Poulikakos et al. reported the appropriateness of creating a person-specific dynamic profile with continuous ECG recording during hemodialysis in determining the risk for sudden cardiac death in hemodialysis patients.[12] They found that PTH was effective in myocardial repolarization. In our study, ECG was performed before and after hemodialysis, and there was no relation with pre-PTH. Kalantzi et al. evaluated the arrhythmogenic potential of hemodialysis sessions and found no significant findings in QRS, QTc interval, and QT dispersion.[18] No effect of electrolytes on QT dispersion and QRS were observed in this study. Calcium and sodium were found to be negatively correlated with post-QTc. Ozportakal et al. examined the effects of serum electrolytes and pH changes on ECG parameters before and after hemodialysis. They emphasized that Tpe, the time between the peak and end of QRS and T wave, increased after hemodialysis and the effect of hypocalcemic patients on the increase in Tpe. They stated that ultrafiltration was associated with mean QTc, and these two parameters were important in repolarization abnormalities.[19] In this study, there was no relationship between calcium and ECG waves, but post-dialysis QTc was found to be negatively significant in multiple regression analysis; its negative correlation with pre-weight and positive correlation with post-weight in volume evaluation was compatible with this study.

Chen et al. reported that the decreasing effect of hyperuricemia on heart rate developed in hemodialysis patients with autonomic dysfunction due to insulin resistance. They also mentioned that it reflects the impaired sympathovagal balance in hemodialysis patients and the potential effects of lowering uric acid levels in lowering heart rate.[20] In this study, the negative correlation of post-RR and pre-QTc with uric acid and the significant effect in univariate linear regression was found to be compatible with the mentioned studies.

Wenjing et al. reported that QTc prolongation was not observed in peritoneal dialysis in contrast to hemodialysis. This suggests that ECG wave change is less in treatment modalities without volume load and short-term shifts of electrolytes.[21] In this study, the correlation of pre- and post-dialysis weights with pre- and post-dialysis QTc suggests the effect of volume load.

Bukhari et al. reported that serum potassium and calcium levels and heart rate strongly affected the T wave.[22] In our study, no correlation was found between RR, which is an expression of heart rate, and serum potassium and calcium. Tarif et al. did not find a relationship between potassium and calcium and ECG after hemodialysis in multiple regression.[23] They reported that a decrease in serum potassium after hemodialysis caused a decrease in the T/R wave ratio on ECG, which was arrhythmogenic. In this study, we did not observe any effect of serum potassium level on post-dialysis RR and found that sodium was negatively correlated with post-dialysis QTc. Szewieczek et al. reported that QRS voltage was positively correlated with various hemodynamic, metabolic, and inflammatory factors, including systolic blood pressure, vitamin B12, sodium, calcium, phosphorus, and e-GFR.[24] In their circadian modeling study of heart rhythm, Wisniowska et al reported that sodium, potassium, calcium electrolyte balance and circadian change were factors affecting the QT circadian change.[25] Fluctuations in sodium during dialysis may cause this effect due to the effect of circadian change of electrolytes on OTc. The negative correlation between sodium and QTc found in this study may be attributed to volume overload in patients receiving end-stage renal replacement therapy.

In this study, the negative correlation between post QTc and sodium and calcium indicate the potential for ventricular arrhythmia, as seen in other studies. Considering that QT prolongation is a non-invasive indicator showing increased susceptibility to ventricular arrhythmias, monitoring and evaluation of electrolytes are important in the prediction of arrhythmia.

### Limitations

This study has several limitations. This study is limited by its retrospective design and single-center setting, which may limit the generalizability of the findings. Additionally, the sample size was relatively small, and not all electrolytes were measured at multiple time points during dialysis. Future studies should consider a larger, multicenter design to enhance generalizability and include continuous ECG monitoring throughout the dialysis session to capture transient changes in cardiac function.

## CONCLUSION

It is known that volume changes and electrolyte shift during hemodialysis have effects on ECG waves and intervals that cannot be determined with precise limits. Chronic exposure renders hemodialysis patients resistant to the cardiac effects of hyperkalemia. Phosphate appears to be affected by its role in energy metabolism. The negative association of sodium with post-QTc appears to affect heart rate through the effect of volume load. The positive correlation of post-dialysis weight with post-dialysis QRS and QTc supports this. In this study, phosphorus had an effect on heart rate, and sodium had an effect on post-dialysis QTc, but statistically, the effects were not very strong. Multifactorial factors on ECG waves and intervals in hemodialysis patients make arrhythmia prediction difficult. This study found significant correlations between post-dialysis ECG changes and serum phosphorus, sodium, calcium and uric acid levels, suggesting that careful monitoring of these electrolytes could help predict arrythmia risk in hemodialysis patients.

## ETHICAL DECLARATIONS

**Ethics Committee Approval**: The study was carried out with the permission of Mardin Artuklu University Non-invasive Clinical Research Ethics Committee (Date: 03.05.2023, Decision No: 2023/5-10).

**Informed Consent:** Informed consent was obtained from all subjects involved in the study.

**Referee Evaluation Process:** Externally peer-reviewed.

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