



Evaluating the effect of thyroid disorders in hemodialysis patients

Mahshad Rahimi¹, Hamid Reza Samimagham², Ali Salimi Aasl¹, MohammadHosein Sheybani-Arani¹, Fatemeh Khajavi-Mayvan¹, Elham Boushehri³, Ladan Hajiabdolrassouli⁴, Mitra Kazemi Jahromi^{4*}

¹ Student Research Committee, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

² Cardiovascular Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

³ Department of Medical Education, School of Health, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

⁴ Endocrinology and Metabolism Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

***Corresponding author:** Mitra Kazemi Jahromi, **Address:** Endocrinology and Metabolism Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran, **Email:** mitra.kazemijahromi@gmail.com, **Tel:** +98

Abstract

Background & Aims: The thyroid gland, a small butterfly-shaped organ in the neck, regulates the body's metabolism. Disruptions in its function can lead to various health issues, including fatigue, weight changes, and cardiovascular problems. In hemodialysis patients, thyroid function is even more crucial. Hemodialysis, a treatment for kidney failure, filters waste and excess fluid from the blood, potentially affecting various bodily systems, including the endocrine system. This study examines the effect of thyroid function on hemodialysis.

Materials & Methods: In this descriptive-analytical study, dialysis patients were classified into three groups: hypothyroid, hyperthyroid, and euthyroid. The levels of thyroid and parathyroid hormones, serum electrolytes, clinical symptoms, laboratory results, and blood pressure of the patients in these groups were compared.

Results: There was no significant difference between the number of dialysis sessions and thyroid function. The serum calcium level was significantly higher in hypothyroid patients than in euthyroid and hyperthyroid patients. There were no significant differences in weight changes before and after dialysis considering the participants' sex and age ($P = 0.227$ and $P = 0.457$). Moreover, there were no significant differences in the number of dialysis sessions ($P = 0.508$), systolic ($P = 0.419$), and diastolic blood pressure ($P = 0.559$), or in the serum level of parathormone in patients with different thyroid functions ($P = 0.103$). However, the serum level of phosphorus was significantly higher in hyperthyroid patients than in normal patients and lower than in hypothyroid patients ($P = 0.049$). The hemoglobin concentration was higher in hyperthyroid patients than in other groups ($P = 0.021$).

Conclusion: The changes in calcium, hemoglobin, and parathormone levels in hemodialysis patients with different thyroid function statuses showed significant differences. These differences are believed to be caused by high bone metabolism in dialysis patients. Evaluating these parameters in dialysis patients is recommended, highlighting the need for regular thyroid function screening among these patients.

Keywords: Thyroid, Hemodialysis, Ferritin

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Introduction

Chronic kidney disease (CKD) is defined as a decrease in glomerular filtration rate or an increase in urinary albumin excretion, and has emerged as a significant public health concern. The global prevalence of this disease is estimated at 8-16%. CKD is associated with increased overall mortality, particularly due to cardiovascular diseases, and can lead to progression of kidney damage, acute kidney injury, reduced cognitive ability, anemia, mineral and bone disorders, and fractures. Notably, thyroid dysfunction is more prevalent in patients with end-stage renal disease (ESRD) and CKD (1, 2).

Various modalities of dialysis are used to manage these patients, depending on their condition and the physician's assessment. Research indicates that kidney diseases, including associated metabolic acidosis, can cause thyroid gland dysfunction. Importantly, correcting chronic metabolic acidosis has been shown to reduce mortality rates in these patients. Furthermore, chronic or end-stage renal failure can itself cause thyroid dysfunction (3, 4).

The deficiency of trace elements, such as selenium, commonly observed in hemodialysis patients and those with chronic uremia, not only affects the regular activity of the thyroid gland but also acts as a stimulating factor for autoimmune thyroid disease. Selenium deficiency has been associated with goiter and thyroid nodules (5, 6). Additionally, the accumulation of excess iodine from dietary sources in patients undergoing dialysis treatment can lead to functional defects of the thyroid gland and hypothyroidism (7). It is crucial to note that hypothyroidism is also associated with increased mortality rates in dialysis patients and can exacerbate kidney failure by reducing cardiac output, decreasing vasodilator production, and increasing tubuloglomerular feedback (8).

Moreover, patients with less frequent hemodialysis sessions per week have been found to have higher levels of thyroid-stimulating hormone (TSH), which is associated with greater resistance to erythropoietin. Interestingly, TSH levels demonstrate an inverse relationship with dialysis adequacy, serum albumin

levels, hemoglobin levels, and the erythropoietin resistance index (ERI) (9).

The aim of this study is to investigate the impact of thyroid function on various clinical parameters in patients with kidney disease. By examining these relationships, we seek to develop strategies for the early diagnosis of thyroid disorders and the prevention of associated complications in this patient population.

Materials and Methods

Participants and design:

This descriptive-analytical study was conducted on 170 patients aged 14 years and older referred to the hemodialysis center of our hospital in 2022. Eligible participants had been undergoing regular dialysis for at least one year. Convenience sampling was employed for participant selection.

Exclusion criteria were as follows:

- History of malignancy, hypercalcemia, autoimmune diseases such as lupus, and type 1 diabetes
- Significant weight changes (more than 10% loss or gain in 6 months)
- Use of medications affecting thyroid function (such as lithium, amiodarone, corticosteroids, carbamazepine, and phenytoin)

After obtaining informed consent, patient information was extracted from medical records using a researcher-developed checklist. Measured variables included blood pressure, weight, and thyroid function tests (FT3, FT4, and TSH). Additionally, monthly measurements of ferritin, PTH, calcium, and phosphorus levels were analyzed.

Patients were classified into three groups based on thyroid function: hypothyroid, hyperthyroid, and euthyroid. Hypothyroidism was defined as TSH levels higher than 5 mIU/L and free T4 levels below 130 pg/dL. Hyperthyroidism was defined as free T4 levels above 2.8 ng/dL or free T3 levels above 450 pg/dL, accompanied by TSH levels below 0.5 mIU/L (1, 2). Patients not meeting these criteria were classified as euthyroid.

Measurements:

Data collected included thyroid and parathyroid hormone levels, thyrotropin, serum calcium and phosphorus levels, systolic and diastolic blood pressure, weight changes during dialysis, hemoglobin concentration, erythropoietin levels, serum ferritin, patient weight, and dialysis frequency and duration. These parameters are routinely recorded monthly in patients' files at this center.

Patient weights were recorded at each thyroid function sampling for two consecutive months. Blood samples were collected monthly for two consecutive months. After centrifugation, thyroid function tests were performed using the fluorescent immunoassay method with the VIDAS® Thyroid Panel medical diagnosis kit and device. The VIDAS® multiparametric

immunoassay system for medium throughput was utilized for these analyses.

Statistical analysis:

Data analysis was performed using SPSS software. ANOVA, regression analysis, Kolmogorov-Smirnov test, Kruskal-Wallis test, Fisher's exact test, paired T-test, and chi-squared test were used to analyze the results and compare differences between groups. A $P < 0.05$ was considered statistically significant.

Results

This study investigated thyroid function in 170 hemodialysis patients. The sample comprised 82 women (48.2%) and 88 men (51.8%), with a mean age of 46.54 ± 11.14 years. The average number of dialysis sessions was 3.12 ± 0.85 times per week (Table 1).

Table 1. Demographic characteristics

Variables		Frequency	Percentage
Gender	Male	88	51.8
	Female	82	48.2
		Mean	Standard deviation
Age (Year)		46.54	11.14
Weight (Kg)		70.61	10.28
Number of dialysis sessions		3.12	0.85
The number of years of dialysis	Under five years	97	57.0
	Above five years	73	43.0
Monthly serum calcium (mg/dL)		8.66	1.32
Monthly serum phosphorus (mg/dL)		5.27	1.29
Hemoglobin (g/dL)		11.31	1.16
T3 ($\mu\text{g/dL}$)		5.16	1.81
T4 ($\mu\text{g/dL}$)		11.95	4.06
TSH (mIU/L)		2.16	1.01
Systolic blood pressure (mmHg)		14.15	2.23
Diastolic blood pressure (mmHg)		8.8	1.47
Ferritin (ng/mL)		312.44	188.40
Parathyroid hormone (pg/mL)		412.5	234.17
Thyroid function	Hyperthyroidism	3	1.76
	Euthyroidism	158	92.94
	Hypothyroidism	9	5.30
Weight difference before and after (Kg)		2.76	0.71

TSH (thyroid stimulating hormone)

Thyroid Function Distribution:

Of the 170 patients, 158 (92.94%) were euthyroid, 9 (5.30%) were hypothyroid, and 3 (1.76%) were hyperthyroid.

Age and Thyroid Function:

The Kruskal-Wallis test revealed statistically significant differences in mean age across thyroid function groups ($p < 0.05$). Hypothyroid patients were significantly older compared to both hyperthyroid and euthyroid patients.

Gender and Thyroid Function:

Fisher's exact test showed no statistically significant difference in gender distribution across thyroid function

groups.

Weight and Thyroid Function:

Analysis of variance (ANOVA) showed no statistically significant differences in initial weight across thyroid function groups, regardless of gender or age group (15-39, 40-55, and 56-70 years).

Weight Changes and Thyroid Function:

The Kruskal-Wallis test revealed no statistically significant differences in weight changes across thyroid function groups ($p = 0.227$), neither in the overall sample nor when stratified by gender or age group (Table 2).

Table 2. Comparison of mean and standard deviation of patient weight changes (before and after starting dialysis) in both sexes and age groups based on thyroid function

		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	statistics	Meaningful
The difference in the patient's weight (before and after the start of dialysis) (Kg)	Male	0.51	2.46	0.69	2.75	-	1.9	3.238	0.198
	Female	0.62	2.32	0.74	2.84	0.56	2.7	1.347	0.510
	Total	0.53	2.4	0.71	2.79	0.61	2.43	2.963	0.227
		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
The difference in the patient's weight (before and after the start of dialysis) (Kg)	Age (year)								
	39-15	-	-	0.74	2.84	-	2.3	0.715	0.398
	55-40	0.57	2.46	0.71	2.86	0.84	2.5	1.218	0.544
	70-56	-	-	0.54	2.32	0.68	2.58	0.554	0.457

Dialysis Frequency and Duration:

The Kruskal-Wallis test showed no statistically significant differences in dialysis frequency or duration

across thyroid function groups ($p = 0.508$), regardless of gender or age group (Table 3).

Table 3. Comparison of the mean and standard deviation of the number of dialysis times and years of dialysis in both genders and age groups based on thyroid function

		hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Number of dialysis sessions	Male	1.22	3	0.84	3.04	-	3	0.069	0.966
	Female	0.57	2.5	0.85	3.25	0.7	3.5	4.06	0.131
	Total	0.97	2.77	0.85	3.14	0.57	3.33	1.35	0.508
Years of dialysis	◦Years <	0.53	3.2	0.76	3.3	0.15	3.8	1.22	0.933
	◦Years ≥	0.46	7.3	0.71	7.8	0.28	8.1	3.21	0.761
	Total	0.51	5.25	0.74	5.055	0.17	5.95	2.11	0.877
		Hypothyroidism		Normal		Hyperthyroidism		Test	
Age (year)		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Number of dialysis sessions	39-15	-	-	0.85	3	-	4	1.85	0.173
	55-40	0.7	3	0.88	3.13	0	3	0.194	0.908
	70-56	-	-	1.29	2.5	0.75	3.31	1.899	0.168

Calcium Levels:

The Kruskal-Wallis test revealed statistically significant differences in mean calcium levels across thyroid function groups ($P = 0.001$). Hypothyroid patients had significantly higher calcium levels compared to euthyroid patients ($p = 0.0001$). This pattern was consistent in both genders and in the age groups 40-55 and 56-70 years (Table 4).

Phosphorus Levels:

Statistically significant differences were observed in mean phosphorus levels across thyroid function groups ($P = 0.049$). Hypothyroid patients had significantly

higher phosphorus levels compared to euthyroid patients ($P = 0.017$). However, these differences were not significant when stratified by gender or age group (Table 4).

Hemoglobin Levels:

ANOVA indicated statistically significant differences in mean hemoglobin levels across thyroid function groups ($P = 0.021$). Hypothyroid patients had significantly lower hemoglobin levels compared to euthyroid patients ($P = 0.017$). This difference was particularly notable in the 40-55 age group ($P = 0.01$) (Table 4).

Table 4. Comparison of mean and standard deviation of calcium, phosphorus and hemoglobin in both genders in age groups based on thyroid function.

		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Calcium (mg/dL)	Male	0.28	10.26	1.39	8.5	-	8.5	8.10	0.017
	Female	0.27	10.12	1.22	8.65	0.42	8.7	6.75	0.034
	Total	0.27	10.2	1.31	8.57	0.32	8.63	14.998	0.001

	Age	Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Calcium (mg/dL)	39-15	-	-	1.57	8.35	-	8.4	0.245	0.620
	55-40	0.19	10.08	1.3	8.78	0.35	8.75	6.783	0.034
	70-56	-	-	0.31	10.35	0.96	8.26	10.214	0.001
	Age	Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Phosphorus (mg/dL)	Male	0.47	6.34	1.34	5.22	-	6.4	5.19	0.074
	Female	0.54	5.97	1.24	5.20	2.75	5.25	1.67	0.433
	Total	0.50	6.17	1.29	5.21	2.05	5.63	6.032	0.049
	Age	Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Phosphorus (mg/dL)	39-15	-	-	1.17	5.51	-	7.2	1.926	0.165
	55-40	0.67	6.22	1.35	5.14	2.19	4.85	3.184	0.204
	70-56	-	-	0.28	6.12	1.24	5.12	2.938	0.087
	Age	Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Hemoglobin (g/dL)	Male	0.20	10.34	1.12	11.3	-	11	1.834	0.166
	Female	0.20	10.17	1.24	11.44	0.84	11.7	2.144	0.124
	Total	0.21	10.26	1.17	11.36	0.72	11.46	3.962	0.021
	Age	Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Hemoglobin (g/dL)	39-15	-	-	0.87	10.75	-	12.3	3.029	0.091
	55-40	0.15	10.16	1.08	11.62	0.07	11.05	4.805	0.01
	70-56	-	-	0.21	10.4	1.43	11.31	1.567	0.219

Blood Pressure:

The Kruskal-Wallis test showed no statistically significant differences in systolic or diastolic blood pressure across thyroid function groups, regardless of gender or age group (Table 5).

Ferritin Levels:

No statistically significant differences were observed in mean ferritin levels across thyroid function groups in the overall sample. However, in the 56-70 age group, hyperthyroid patients had significantly lower ferritin levels compared to euthyroid patients ($P = 0.042$) (Table 5).

Table 5. Comparison of mean and standard deviation of systolic blood pressure, diastolic blood pressure, and ferritin in both genders in age groups based on thyroid function.

		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Systolic blood pressure before dialysis (cmHg)	Male	2.70	14.6	2.33	14.04	-	10	2.80	0.246
	Female	2.21	15.25	2.10	14.22	2.12	14.5	0.55	0.760
	Total	2.36	14.88	2.21	14.13	3	13	1.73	0.419
	Age	Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Systolic blood pressure before dialysis (cmHg)	39-15	-	-	2.21	14.14	-	16	0.903	0.342
	55-40	3.04	14.4	2.31	14.06	2.12	11.5	2.555	0.279
	70-56	-	-	1.29	15.5	2	14.28	1.493	0.222
		hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Diastolic blood pressure before dialysis (cmHg)	Male	1.64	8.8	1.54	8.78	-	6	2.296	0.317
	Female	1.29	9.5	1.39	8.81	1.41	9	0.741	0.690
	Total	1.45	9.11	1.47	8.79	2	8	1.162	0.559
	Age	Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	mean	Standard deviation	mean	Standard deviation	mean	Statistics	meaningful
Diastolic blood pressure before dialysis (cmHg)	39-15	-	-	2.21	14.14	-	16	0.833	0.362
	55-40	1.92	8.8	1.57	8.74	1.41	7	2.445	0.294
	70-56	-	-	0.57	9.5	1.25	8.88	1.044	0.307

		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Ferritin (ng/mL)	Male	215.01	446	197.7	312.68	-	340	2.91	0.233
	Female	164.59	322.5	181.38	302.5	31.81	312.5	0.494	0.781
	Total	193.67	391.11	189.49	307.78	27.53	321.66	3.007	0.222
		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Age	39-15	-	-	231.27	361.47	-	335	0.039	0.843
	55-40	80.43	278	160.34	297.07	35.35	315	0.833	0.659
	70-56	-	-	208.38	532.5	208.64	282.85	1.15	0.042

Parathyroid Hormone Levels:

The Kruskal-Wallis test showed no statistically significant differences in mean parathyroid hormone levels across thyroid function groups in the overall

sample. However, in the 56-70 age group, hyperthyroid patients had significantly higher parathyroid hormone levels compared to euthyroid patients ($P = 0.009$) (Table 6).

Table 6. Comparison of the mean and standard deviation of parathyroid hormone in both sexes and age groups based on thyroid function.

		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Parathyroid hormone (ng/mL)	Male	110.13	226	246.33	425.42	-	305	3.37	0.185
	Female	151.73	297.5	229.51	421.11	56.56	305	1.47	0.480
	Total	126.96	257.77	237.64	423.35	40	305	4.55	0.103
		Hypothyroidism		Normal		Hyperthyroidism		Test	
		Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Statistics	Meaningful
Age	39-15	-	-	207.75	255.29	-	345	0.981	0.322
	55-40	77.65	326	234.43	458.98	28.28	285	2.731	0.255
	70-56	-	-	132.25	172.5	199.05	496	6.854	0.009

Discussion

The present study was designed and implemented to investigate thyroid function in hemodialysis patients. We analyzed medical information of patients undergoing hemodialysis, including thyroid hormone levels (T3, T4, and TSH), electrolytes (calcium, phosphorus), hemoglobin, ferritin, systolic and diastolic blood pressure, weight changes during dialysis, and dialysis frequency.

Kidney diseases, particularly those associated with metabolic acidosis, can disrupt thyroid gland function. Correcting chronic metabolic acidosis has been shown to reduce mortality rates in these patients. However, it is important to note that some medications used in treating chronic kidney disease or end-stage renal failure may also affect thyroid function (1, 2).

In chronic renal failure and hemodialysis, thyroid function and its regulatory hormones may be altered. For

instance, the peripheral conversion of T4 to T3 is impaired in kidney diseases, resulting in decreased T3 levels. Additionally, these patients exhibit decreased hormone content in thyroid tissue and increased accumulation in the gland (1).

Hypothyroidism influences bone metabolism and, consequently, serum calcium levels (2, 3). Studies have shown that hypothyroidism typically leads to decreased serum calcium levels, with subsequent increases in parathyroid hormone and vitamin D concentrations (4, 5). Rarely, hypercalcemia has been reported in hypothyroid patients taking calcium supplements (6).

Our findings revealed significant variations in calcium levels across different thyroid states. Interestingly, hypothyroid patients showed higher serum calcium levels compared to hyperthyroid and euthyroid patients in both sexes. This unexpected finding warrants further investigation. Age-specific analysis revealed that these differences were most prominent in patients aged 40 to 55 years.

These results contrast with those reported by Rhee et al. (8), who found no significant differences in serum calcium levels among euthyroid, hypothyroid, and all patients. This discrepancy may be attributed to differences in patient characteristics, particularly the higher average age in our study population.

Regarding phosphorus levels, our study found significant differences among patients with different thyroid functions. This aligns with Zoccali et al. (7), who reported higher calcium-phosphate product in patients with elevated T3 levels.

Contrary to expectations based on the known effects of hypothyroidism on bone turnover and parathyroid hormone levels (8, 9), our study did not find significant differences in parathormone levels across all thyroid function groups. However, age-specific analysis revealed that in the 56-70 year age group, euthyroid patients had lower parathormone levels compared to hyperthyroid patients. These findings differ from Rhee et al. (10), who reported higher parathormone levels in euthyroid patients.

As anticipated, hemoglobin levels were lowest in hypothyroid patients, intermediate in euthyroid patients,

and highest in hyperthyroid patients. This suggests that the relationship between thyroid function and hemoglobin levels in hemodialysis patients mirrors that observed in the general population. However, we found no significant differences in ferritin levels across thyroid function groups, contrasting with some previous studies (11, 12).

Our study found no significant relationship between thyroid function status and blood pressure in hemodialysis patients, consistent with findings from Zoccali et al. (7).

The role of trace elements, particularly selenium, in thyroid function among hemodialysis patients is noteworthy. Selenium deficiency, common in this population, may contribute to thyroid dysfunction and autoimmune thyroid disease (3, 4). Additionally, iodine accumulation in dialysis patients can lead to hypothyroidism and thyroid gland dysfunction (5).

Hypothyroidism has been associated with increased mortality in dialysis patients and may exacerbate kidney dysfunction through various mechanisms (6). Furthermore, less frequent dialysis has been linked to higher TSH levels and increased erythropoietin resistance (7).

Our finding of no significant difference in ferritin levels across thyroid function groups contrasts with some previous studies (12, 13). These discrepancies may be due to differences in study populations, geographical factors, or methodological approaches.

In our study, hypothyroidism was the most common thyroid dysfunction, consistent with findings from Lebikowska et al. (16) and Sennesael et al. (14).

We found no statistically significant gender differences in thyroid function distribution, contrary to some previous studies suggesting gender-specific differences in dialysis outcomes (18, 19).

Conclusion

Our study reveals significant differences in calcium, phosphorus, hemoglobin, and parathormone levels among hemodialysis patients with different thyroid function statuses. These differences may be attributed to the complex interplay between thyroid function, bone

metabolism, and the uremic state in dialysis patients. We recommend careful evaluation of these parameters in dialysis patients, considering clinical findings and individual patient factors.

Limitations of the study

The relatively low frequency of hypothyroid patients in our sample may have affected the statistical power of our analyses. Future studies should focus on this subgroup to better understand their unique characteristics and management needs. Additionally, the cross-sectional nature of our study and single time-point measurements limit our ability to control for potential confounding factors. Prospective studies with longitudinal data collection would provide more robust insights into the relationships between thyroid function and other physiological parameters in hemodialysis patients.

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Ethical statement

The research was conducted in accordance with the principles of the Declaration of Helsinki. The Ethics Committee of Hormozgan University of Medical Sciences approved this study. The institutional ethical committee at Hormozgan University of Medical Sciences accepted all study protocols (IR.HUMS.REC.1398.476). Accordingly, written informed consent was obtained from all participants before any intervention. In addition, ethical issues (including plagiarism, data fabrication, and double publication) were entirely observed by the authors.

Data availability

The data sets used during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceptualization: MR, HRS, MKJ, Methodology: MKJ, ASA, FKM, EB, Investigation: EB, LH, MR,

MSA, Writing—Original Draft Preparation: FKM, LH, ASA, MSA, Writing—Review and Editing: FKM, MKJ, ASA, MSA.

Conflict of interest

The authors declare no conflict of interest.

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