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Relationship between erythropoietin resistance and secondary hyperparathyroidism in dialysis patients

SUMMARY

of dissertation for the award of scientific and educational degree "Doctor"

Supervisor:

Prof. Dr. Svetla Vasileva Staykova, MD

Varna, 2021

The dissertation contains 146 standard pages and is illustrated with 12 tables, 59 figures and 1 appendix. The literature includes 390 literary sources, of which 3 in Cyrillic and 387 in Latin.

The dissertation was discussed and directed to the defense of the Department of the Second Department of Internal Medicine at the Medical University "Prof. Dr. Paraskev Stoyanov ”- Varna.

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The official defense of the dissertation will take place on January 21, 2022 at ……. in ..... - Varna at an open meeting of the Scientific Jury.

The materials on the defense are available in the Scientific Department of MU - Varna and are published on the website of the Medical University - Varna.

Note: In the abstract the numbers of the tables and figures do not correspond to the numbers in the dissertation.

CONTENTS

ABBREVIATIONS USED

CRP – C-реактивен протеин

CKD – chronic kidney disease

CKD-MBD – chronic kidney disease – mineral bone disorders

eGRF – estimated glomerular filtration rate

ESA – eryhtopoesis stimulating agents

EPO – erythropoietin

ERI – erythropoietin resistance index

ESRD – end stage renal disease

FGF-23 – Fibroblast Growth Factor 23

HIF – Hypoxia Inducible Factor

HIF –PHIs - Hypoxia Inducible Factor – Prolyl Hydroxilase Inhibitors

HR-QOL – health-related quality of life

KDIGO – Kidney Disease: Improving Global Outcome

KDOQI - Kidney Disease Outcomes Quality Initiative

PTH – parathyroid hormone

SHPT - Secondary hyperparathyroidism

SLE – systemic lupud erythematosus

rHuEPO – recombinant erythropoietin

I. INTRODUCTION

The incidence of chronic kidney disease (CKD) has been rising rapidly in recent years, and this is a serious health problem worldwide. Anemia is a common complication of CKD and reduces quality of life and increases the risk of cardiovascular diseases and mortality, especially in the group of patients with end-stage renal disease (ESRD). There are various causes of renal anemia, such as decreased endogenous erythropoietin (EPO) production, EPO resistance, reduced red blood cell half-life, and bone marrow fibrosis. Secondary hyperparathyroidism (SHPT) is another lesser known but potentially significant cause of anemia in patients with CKD. Parathyroid hormone (PTH) is considered a uremic toxin that inhibits EPO synthesis, shortens red blood cell survival and causes myelofibrosis. Metabolic changes that occur in patients with CKD have a significant impact on mineral and bone metabolism. Deviations in serum calcium, phosphate and PTH levels are a common challenge in the treatment of CKD. These biochemical abnormalities, together with dysregulation of vitamin D metabolism and bone metabolism, are a systemic syndrome called CKD-MBD and are associated with increased morbidity, mortality and adverse effects on patients' quality of life.

Erythropoietin resistance is directly related to the incidence of comorbidity in dialysis patients and is one of the most potent predictors of the risk of cardiovascular events and mortality. Frequent monitoring of laboratory parameters, early identification of the causes of its development and timely treatment are essential to prevent potential complications. Individualization of therapy is recommended - assessing the benefits / risks of continuing treatment with ESA, as well as seeking new therapeutic options in these patients. The main factors for improving the quality of life in this group are the provision of hemodialysis centers with highly qualified medical staff, good collaboration between patients and medical staff, proper care for vascular access.

II. PURPOSE, TASKS, HYPOTHESIS

2.1. Purpose

The aim of this study is to analyze the diagnostic and therapeutic aspects of secondary hyperparathyroidism in patients with CKD and their importance for the development of erythropoietin resistance.

2.2. Tasks

To achieve this goal, we set ourselves the following tasks:

1) Study of the relationship between secondary hyperparathyroidism and erythropoietin resistance in patients with chronic kidney disease.

2) Study of other factors responsible for the development of erythropoietin resistance (serum iron, folic acid, vitamin B12, soluble erythropoietin receptor, antibodies to erythropoietin, adequacy and duration of hemodialysis treatment) in patients with chronic kidney disease.

3) Correlation between some markers of mineral and bone metabolism (including iPTH, iFGF-23) and indicators of erythropoietin resistance (serum iron, folic acid, vitamin B12, soluble erythropoietin receptor, antibodies to erythropoietin, CRP) in patients with chronic kidney disease.

4) Comparison of the results of the treatment of secondary hyperparathyroidism and anemia and the required doses of drugs

5) Study in dynamics of the individual quality of life of patients with secondary hyperparathyroidism and anemia due to CKD.

6) Development of an algorithm for the diagnosis of erythropoietin resistance in patients with CKD.

2.3. Hypothesis

The constellation of diagnostic and therapeutic methods we have developed contributes to elucidating the causes of erythropoietin resistance and their relationship to secondary hyperparathyroidism in patients with chronic kidney disease. For optimal results and improvement of the quality of life it is necessary to apply an individualized approach.

III. MATERIAL AND METHODS

3.1. Subjects and range of the study

The subject of the study are a total of 80 patients with chronic kidney disease, divided into two groups – pre-dialysis patients (30) and patients undergoing hemodialysis treatment (50) from the Clinic of Nephrology and Dialysis at the University Hospital "St. Marina" in Varna, followed clinically and examined by routine methods.

a) Criteria for inclusion of persons

 Persons over 18 years of age with chronic kidney disease

 Persons with CKD in pre-dialysis and dialysis stages receiving short-acting ESA treatment (Epoetin alfa)

 Persons without concomitant malignancies

 Persons not taking ACE inhibitors / ARBs

 Persons who have signed an informed consent

b) Criteria for exclusion of persons

 Persons under 18 years of age

 Persons with concomitant malignancies

 Persons with active bleeding from the gastrointestinal system

 Persons accepting ACE I / ARB

 Persons who have not signed an informed consent

3.2. Research period

The current complex diagnostic-therapeutic study was conducted for a period of 6 months - April - October 2021. The study is carried out in connection with a project funded by the Science Fund at Medical University -Varna.

3.3. Research methodology

3.3.1. Laboratory researches

The following laboratory parameters were studied:

 Complete blood count (including hemoglobin)

 Intact parathyroid hormone (iPTH)

 Intact fibroblast growth factor -23 (iFGF-23)

 Folic acid

 Vitamin B12

 Soluble erythropoietin receptor

 Antibodies to erythropoietin

 Serum iron, TIBC, TSAT

 CRP

 Albumin

 Calcium

 Phosphorus

 Urea reduction ratio (URR)

 Erythropoietin resistance index (ERI)

Indicator	Apparatus	Methods
Hemoglobin	Sysmex XN – 1000	Cyanide-free colorimetric method using Sodium lauryl sulphate (SLS)
Serum Iron	Siemens ADVIA 1800	Colorimetric method with Ferrozine.
TIBC	Siemens ADVIA 1800	Direct method with successive release and uptake of iron
Tranferrin saturation	Siemens ADVIA 1800	Calculated
Albumin	Siemens ADVIA 1800	Colorimetric method with Bromocresol green BCG
Total calcium	Siemens ADVIA 1800	Colorimetric method with Arsenazo III
Phosphate	Siemens ADVIA 1800	Colorimetric method with cPhospomolybdate/UV
Ferritin	Roche Cobas 6000	Electrochemiluminescent immunoassay
PTH	Immulite 2000 XP	Chemiluminescent immunoassay

Hematological analysis - Sysmex XN - 1000 hematological analyzer. Blood counts were determined on a hematological analyzer using the principles of fluorescent flow cytometry using a semiconductor laser and hydrodynamic focusing:

 Blood cells count - with the possibility of impedance and optical method of reading.

 Differential leukocyte count - fluorescence flow cytometry with lateral fluorescent light, front scattered and side scattered light.

 Hemoglobin - cyanide-free colorimetric method using Sodium lauryl sulphate (SLS).

Biochemical parameters - Biochemical analyzer SIEMENS ADVIA 1800:

 Serum Iron - colorimetric method with Ferrozine

 TIBC - direct method with successive release and uptake of iron

 Transferrin saturation - calculated

 Albumin - colorimetric method with Bromocresol green - BCG

 Total calcium - colorimetric method with Arsenazo III

 Phosphorus - colorimetric method with Phospomolybdate / UV

Serum levels of vitamin B12, folic acid, antibodies to erythropoietin (anti-EPO antibodies), human soluble erythropoietin receptor (Human EPOR) and intact fibroblast growth factor-23 (iFGF-23) were examined in all participants using sandwich ELISA

3.3.1.1. Determination of serum levels of vitamin B12

Vitamin B12 levels were determined in blood serum using a Vitamin B12 (VB12) ELISA Kit, catalog number UNDL00079, from ELISA Genie, Dublin, Ireland, with a

sensitivity (detection threshold) of 3.8 ng/ml and a linear range 4,687 - 300 ng/ml. The test material is a serum, taken with a closed serum separation system with Vacutainer SST II Advance gel from Beckton Dickinson. After venipuncture, the blood was left for 30 minutes at room temperature to coagulate, and then the serum was separated by centrifugation for 15 minutes at 1,000 × g and stored at -80 ° C until analysis. The analysis of vitamin B12 was performed according to the manufacturer's protocol. The concentration of vitamin B12 in ng / ml was calculated on the basis of the relevant standards by 5 parametric logistic nonlinear regression using MikroWin software version 4.31.

3.3.1.2. Determination of serum folic acid levels

Folic acid levels were determined in blood serum using a Folic acid (FA) ELISA Kit, catalog number UNEB0032, from ELISA Genie, Dublin, Ireland, with a sensitivity (detection threshold) of 0.12 ng / ml and a linear range 0.312-20 ng/ml. The test material is a serum, taken with a closed serum separation system with Vacutainer SST II Advance gel from Beckton Dickinson. After venipuncture, the blood was left for 30 minutes at room temperature to coagulate, and then the serum was separated by centrifugation for 15 minutes at 1,000 × g and stored at -80 ° C until analysis. The analysis of folic acid was performed according to the manufacturer's protocol. The folic acid concentration in ng/ml was calculated based on the relevant standards using 5 parametric logistic nonlinear regression using GraphPad Prism software version 9.2.0.

3.3.1.3. Determination of serum levels of anti-EPO antibodies

Levels of anti-EPO antibodies were determined in blood serum using a test kit Human anti-EPO antibody (anti-Erythropoietin antibody) ELISA Kit, catalog number HUFI003337, from ELISA Genie, Dublin, Ireland, with sensitivity (detection threshold) 0.469 ng/ml and a linear range of 0.781-50 ng/ml. The test material is a serum taken with a closed serum separation system with Vacutainer SST II Advance gel from Beckton Dickinson. After venipuncture, the blood was left for 30 minutes at room temperature to coagulate, and then the serum was separated by centrifugation for 15 minutes at 1,000 × g and stored at -80 ° C until analysis. The analysis of anti-EPO antibodies was performed according to the manufacturer's protocol. The concentration of anti-EPO antibodies in ng/ml was calculated based on the relevant standards using 5 parametric logistic nonlinear regression using MikroWin software version 4.31.

3.3.1.4. Determination of serum levels of FGF23 (intact)

FGF23 (intact) levels were determined in blood serum using a FGF23 (Intact) ELISA test kit for the quantitative determination of Human Intact FGF 23, catalog number BI-20700, from Biomedica Medizinprodukte., Wien, Austria, with sensitivity on detection) 5.4 pg / ml and a linear range of 0-1600pg/ml. The test material is a serum taken with a closed serum separation system with Vacutainer SST II Advance gel from Beckton Dickinson. After venipuncture, the blood was left for 30 minutes at room temperature to coagulate, and then the serum was separated by centrifugation for 15 minutes at 1,000 × g and stored at -80 ° C until

analysis. The analysis of FGF23 (intact) was performed according to the manufacturer's protocol. The concentration of FGF23 (intact) in pg / ml was calculated based on the relevant standards using 5 parametric logistic nonlinear regression using MikroWin software version 4.31.

3.3.1.5. Determination of human EPOR serum levels

Human EPOR levels were determined in blood serum using a EPOR ELISA kit, catalog number RK00280, from Abclonal Technology Co., Ltd. Wuhan, Hubei, P.R China, with a sensitivity (detection threshold) of 34.1 pg / ml and a linear range of 78-5000 pg/ml. The test material is a serum taken with a closed serum separation system with Vacutainer SST II Advance gel from Beckton Dickinson. After venipuncture, the blood was left for 30 minutes at room temperature to coagulate, and then the serum was separated by centrifugation for 15 minutes at 1,000 × g and stored at -80 ° C until analysis. Human EPOR analysis was performed according to the manufacturer's protocol. The concentration of Human EPOR in pg/ml was calculated based on the relevant standards using 5 parametric logistic nonlinear regression using MikroWin software version 4.31.

3.3.2. Urea Reduction Ratio (URR) is calculated using the formula

$$\mathbf{URR = [(Upre - Upost)/Upre] * 100\%}$$

3.3.3. The erythropoietin resistance index (ERI) is calculated as the average weekly dose of erythropoietin (EPO) per kilogram body weight (wt) (IU / kg / week) divided by the mean hemoglobin (Hb) g/dL:

$$\mathbf{ERI = (EPO/wt)/Hb}$$

3.3.4. The individual quality of life was studied through a specialized guide to quality of life in patients with kidney disease with 36 questions (Kidney Disease Quality of Life - Short Form - 36, KDQOL-36) after modification by S. Staykova (2018) in order to adapt it to the situation in our country. Changes have been made in the texts of individual questions and a questionnaire has been created for patients undergoing hemodialysis. (Application 1)

3.3.5. Statistical methods - for analysis and interpretation of experimental data in order to reveal the nature of the observed phenomena and their interdependencies, object of the present dissertation:

- Dispersion analysis (ANOVA) - the frequency distribution of the considered features is presented as a tables and graphics;
- Variation analysis - to assess the quantitative characteristics of the state of the studied trait. For this purpose, the typical for the given population is established and the influence of the lawfully acting factors is described. It is especially important to characterize the scattering and variation of the signs in order to take into account the influence of random factors.

- Correlation analysis - applied to reveal the causal relationships between individual studied traits.
- Regression analysis - statistical analysis of the results obtained to determine the type and parameters of one or more factors, the results are presented in the form of experimental data.
- Comparative analysis (evaluation of hypotheses);
- Odd ratio analysis (OR)
- Assessment of the reliability of the questionnaire used (Cronbach'α).

Data were statistically processed using SPSS v.20, using descriptive indicators for quantitative and qualitative variables and presented in tables and graphics.

The study was conducted with the permission of Ethics committee of Medical University -Varna with Protocol / Decision № 101 / 24.03.2021, as each participant filled in his own declaration of informed consent.

IV. RESULTS

4.1. Characteristics of the studied patients

80 patients of the Clinic of Nephrology at the University Hospital "St. Marina ”- Varna, divided into two groups: 30 patients with chronic kidney disease in pre-dialysis stage (stage 3-4) and 50 patients with CKD stage 5, on dialysis treatment. The characteristics are presented in table. 1.

Table. 1. Characteristics of the patients

Indicators		Pre-dialysis patients (n=30)	Dialysis patients (n=50)	P value
Age (years)	mean±SD (range)	64.33±13.66 (26-85)	62.32±13.51 (36-88)	0.522
Gender	Male	50.0%	58.0%	0.321
	Female	50.0%	42.0%	
Diagnosis	Diabetic nephropathy	16.7%	20.0%	0.528
	Hypertensive nephropathy	56.7%	44.0%	
	Chronic glomerulonephritis	16.7%	26.0%	
	Chronic tubulointerstitial nephritis	10.0%	6.0%	
	Autosomal dominant polycystic kidney disease	-	4.0%	
Duration of dialysis treatment (in months)	mean±SD (range)	-	46.48±45.58 (1 – 192)	-
Estimated glomerular filtration rate (eGFR)	44-30 ml/min/1.73m^2	90.0 %	-	-
	29-15 ml/min/1.73m^2	10.0 %	-	-

The results of the comparative analysis show that no difference is found in terms of age, gender and diagnosis in the two groups, which gives reliability to the results of subsequent analyzes of indicators.

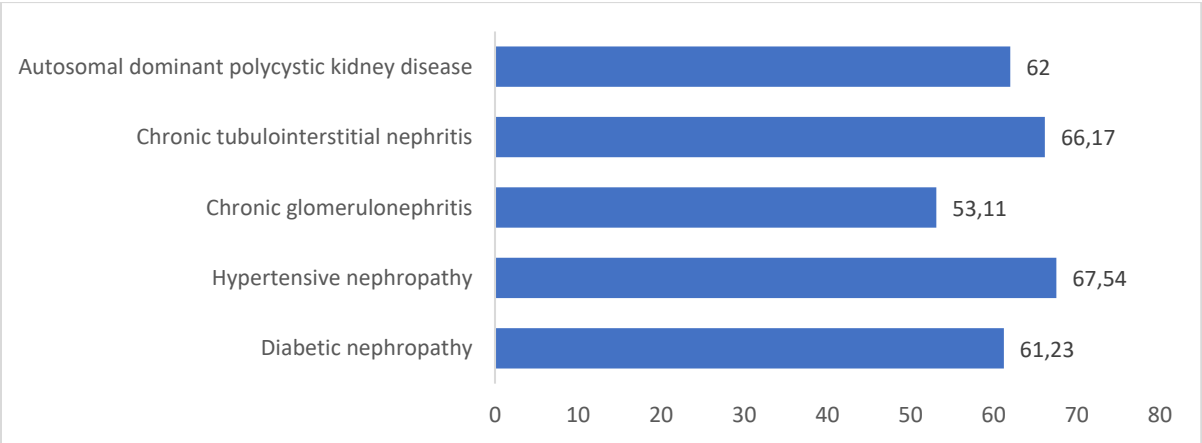

Fig. 1. Average age of patients according to diagnosis

The only difference found was in the diagnosis and age of the patients (p = 0.007), with the oldest being patients with diabetic and hypertensive nephropathy (69.5 years) and the youngest those with chronic glomerulonephritis (53.1 years). (Fig. 1). This could be explained by the aging population and the high incidence of socially significant diseases - diabetes and hypertension.

4.2. Study of the relationship between secondary hyperparathyroidism and erythropoietin resistance in patients with chronic kidney disease

On table 2 are presented the characteristics of patients according to the studied indicators of secondary hyperparathyroidism and erythropoietin resistance.

A comparative analysis is performed and the results are presented in table 2, it was found that there was a significant difference in the levels of iPTH, ERI, phosphorus and iFGF-23, where the values were significantly higher in patients in the dialysis group. On the other hand, albumin levels are significantly higher in patients in the pre-dialysis group.

Regarding to hemoglobin and calcium levels, no significant difference was found between the two studied groups.

Table 2. Characteristics of patients according to the studied indicators

Indicator		Pre-dialysis patients (n=30)	Dialysis patients (n=50)	P value
Parathyroid hormone (iPTH)	mean±SD (range)	345.71±249.48 (77-1305)	622.94±735.48 (10.90-2500)	0.05
Hemoglobin	mean±SD (range)	90.63±11.36 (63-113)	92.38±11.39 (62-110)	0.508
Erythropoietin resistance index (ERI)	mean±SD (range)	15.03±5.28 (5.95-25.39)	19.32±6.47 (10.60-41.00)	0.003
Calcium	mean±SD (range)	2.17±0.24 (1.70-2.65)	2.45±1.02 (1.45-9.06)	0.142
Phosphorus	mean±SD (range)	1.49±0.46 (0.7-2.80)	1.95±0.67 (0.57-3.26)	0.002
Albumin	mean±SD (range)	38.81±6.56 (19.80-48.00)	34.82±4.97 (22.0-48.50)	0.003
iFGF23	mean±SD (range)	517.93±718.43 (12.88-2247.92)	1392.75±707.77 (166.44-2236.25)	< 0.001

The mean value of iPTH was 518.98 ± 620.09 pg/ml (10.9 - 2500pg ml) (Fig. 2), with the mean value of the 25th percentile being 136.5 pg/ml, the 50th being 280.0pg /ml and the 75th percentile is 621.5 pg/ml.

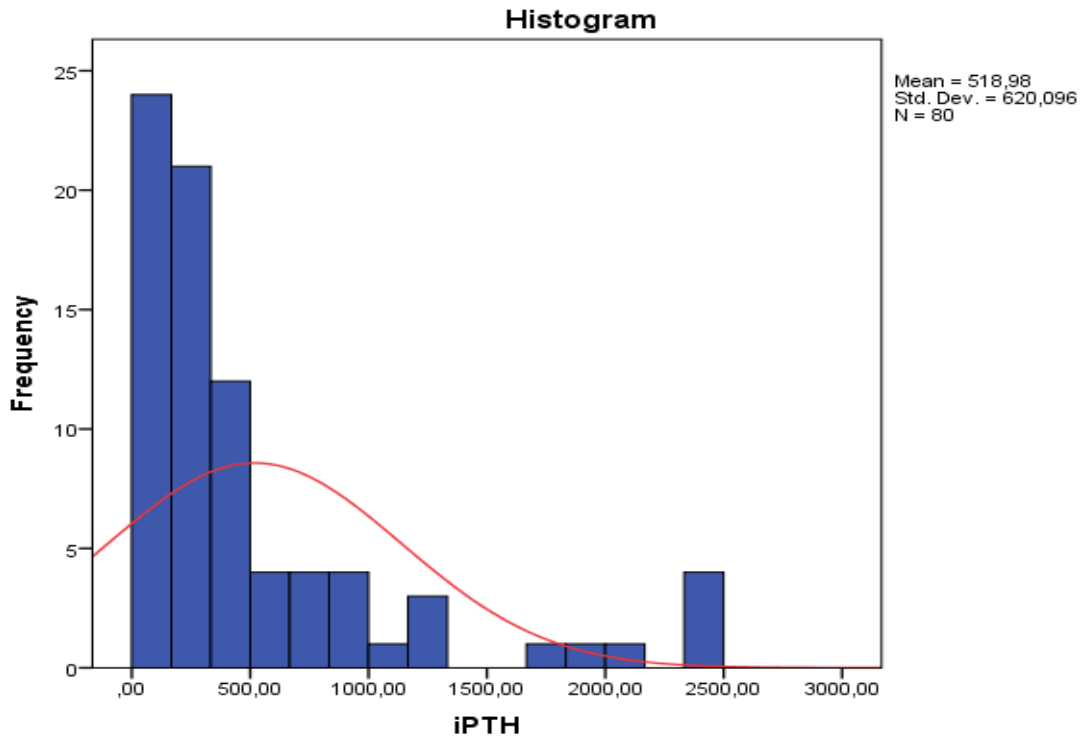

Fig. 2. Distribution of iPTH

There is a significant difference (p = 0.05) and low dependence between the studied group and iPTH (r = 0.218; p = 0.05), as the values of iPTH in dialysis patients were significantly higher (Fig. 3).

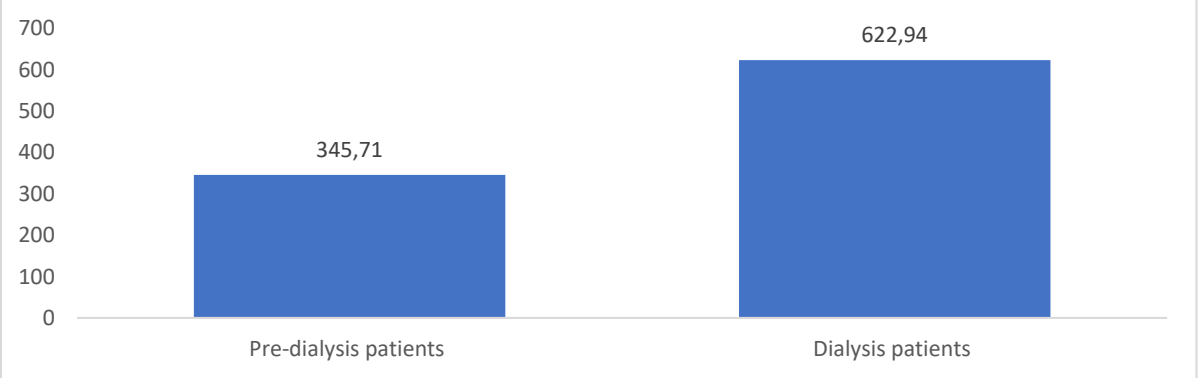

Fig. 3. Comparative analysis of iPTH values according to the studied group

An inverse relationship was found between iPTH and the age of the patients (r = -0.217; p = 0.05), with decreasing iPTH levels with age (Fig. 4). These results become even more pronounced with the duration of dialysis treatment.

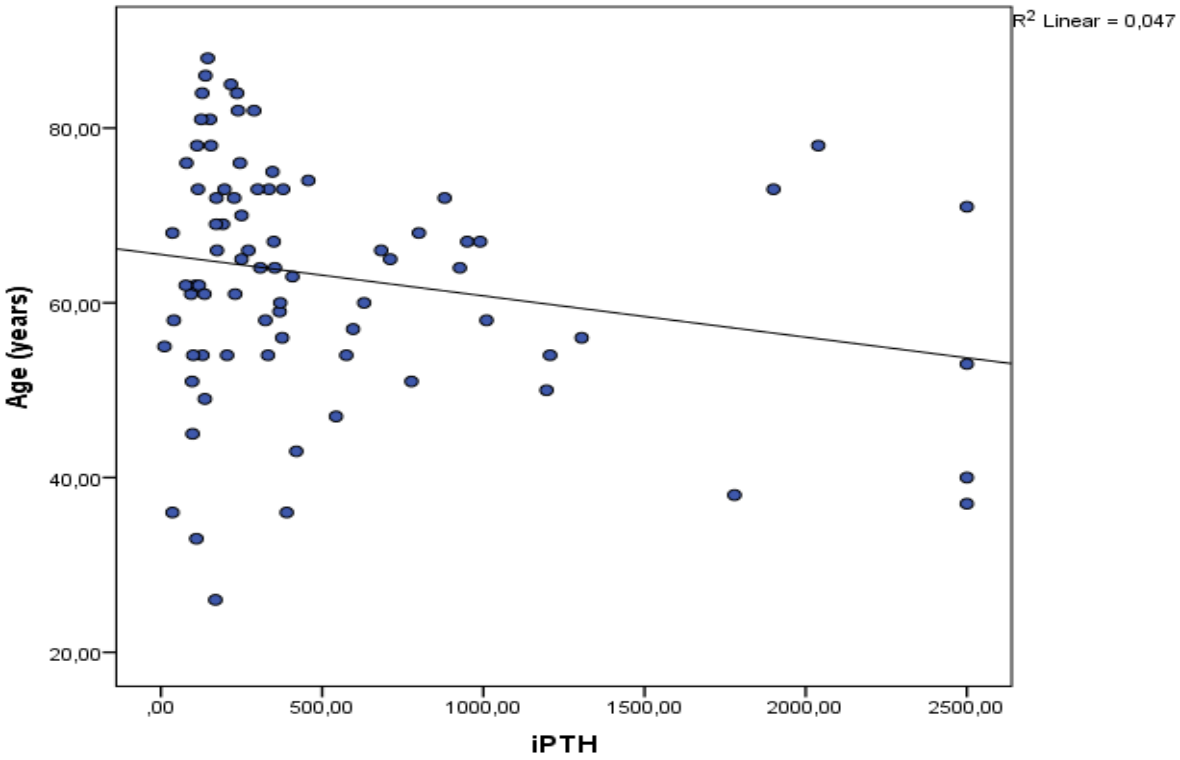

Fig. 4. Dependence between iPTH and age of patients

IPTH levels between 136.5 - 280 pg/ml were found in the oldest patients (71.2 years) and iPTH values> 621.5 pg/ml in the youngest patients (59.4 years).

Although lower iPTH values were observed in men (476.08pg/ml) than in women (571.41pg/ml), no significant difference was found.

There was a significant difference (p <0.001) and a moderate dependence in iPTH levels according to the diagnosis (r = 0.343; p = 0.002) (Fig. 5).

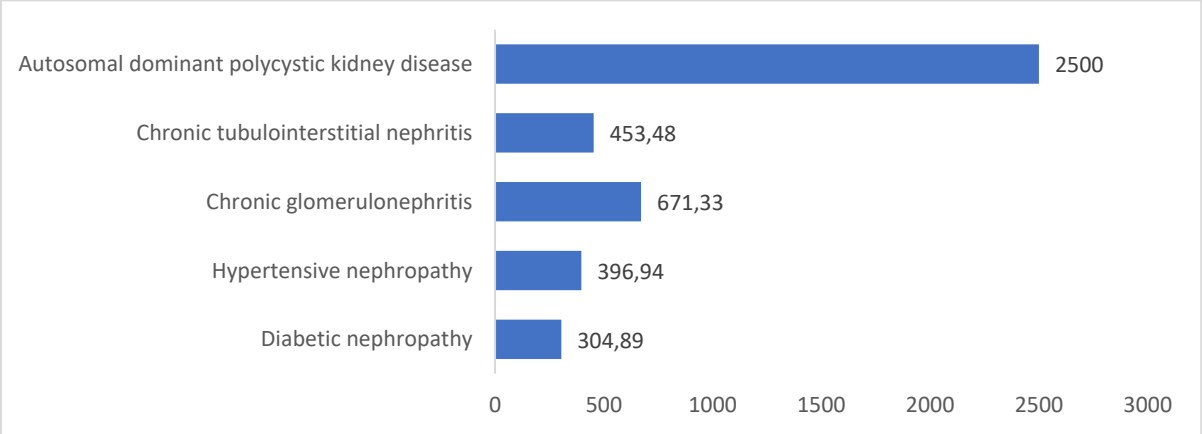

Fig. 5. Mean iPTH values by diagnosis

No correlation was found between iPTH and ERI (Fig. 6). No dependence was also observed for iPTH and calcium, phosphorus, hemoglobin and iFGF-23 levels.

A comparative analysis of ERI values compared to iPTH values in pre-dialysis and dialysis patients revealed a significant difference ($p = 0.003$) (Fig. 7).

Significant difference was found with in the comparison of the levels of iPTH and phosphorus in the two studied groups ($p = 0.002$) (Fig. 8).

Another significant difference was found when comparing the levels of iPTH and iFGF-23 in the two studied groups ($p < 0.001$) (Fig. 9).

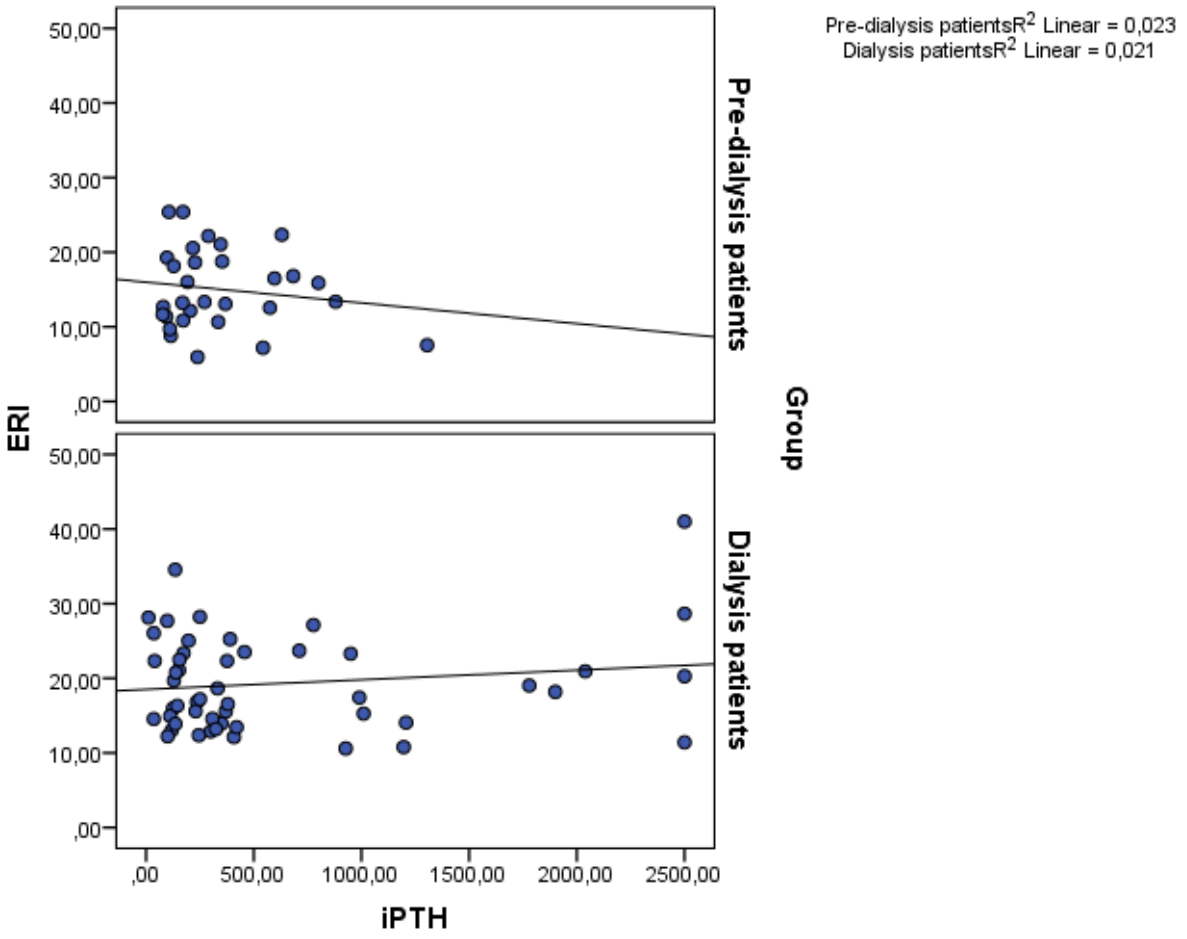

Fig. 6. Dependence between iPTH and ERI

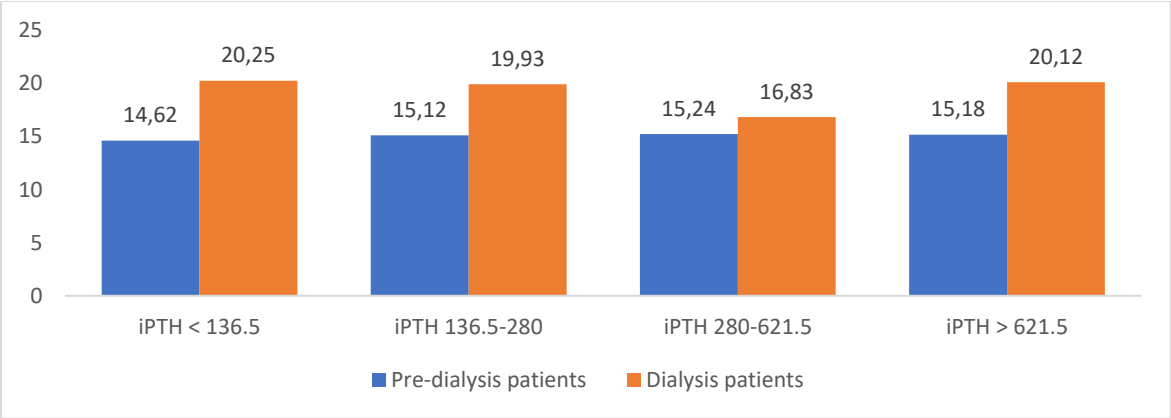

Fig. 7. Comparative analysis between iPTH and ERI according to the studied groups

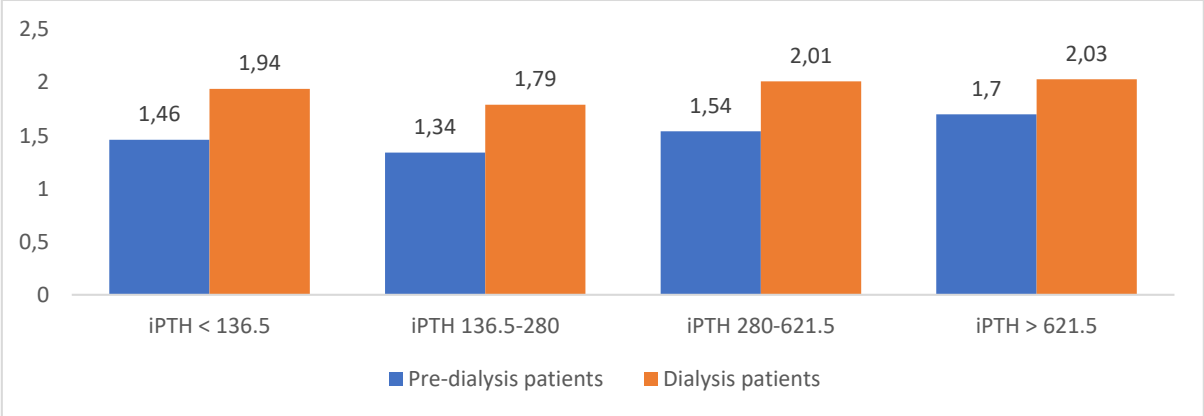

Fig. 8. Comparative analysis between iPTH and phosphorus according to the studied groups

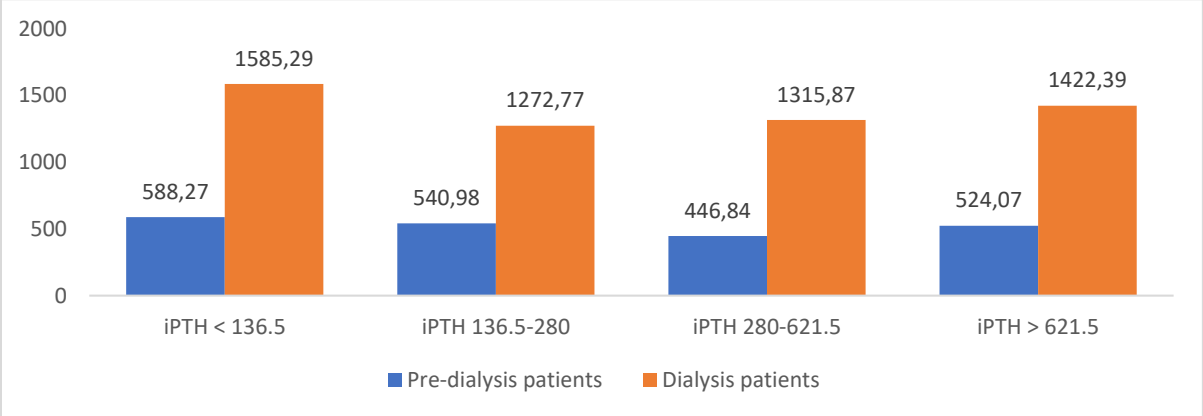

Fig. 9. Comparative analysis between iPTH and iFGF-23 according to the studied groups

A comparative analysis of the results found no difference between iPTH and hemoglobin levels.

A weak to moderate inverse relationship between iPTH and eGRF was found in the group of pre-ialysis patients (r = -0.292; p <0.05) (Fig. 10).

There was a significant difference in albumin levels according to iPTH between the two studied groups (p = 0.003), as in the pre-dialysis group there was an increase in albumin levels with increasing of iPTH levels, while in the dialysis group the opposite trend was found. 11).

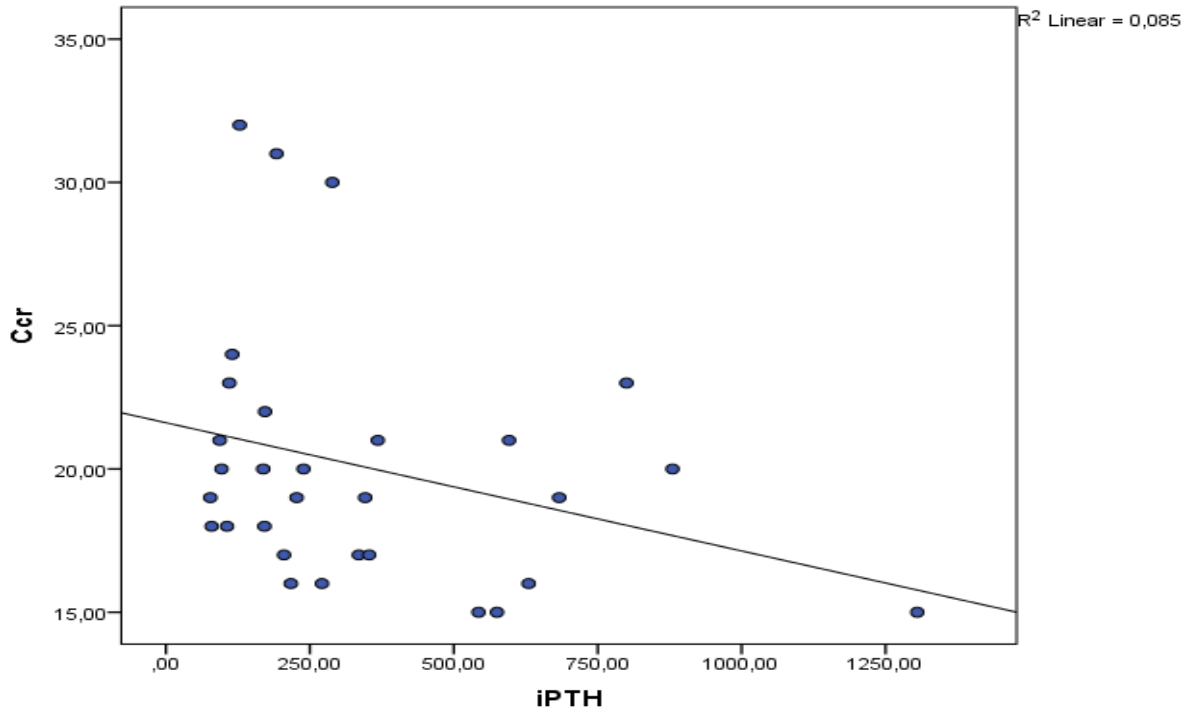

Fig. 10. Dependence between iPTH and eGFR

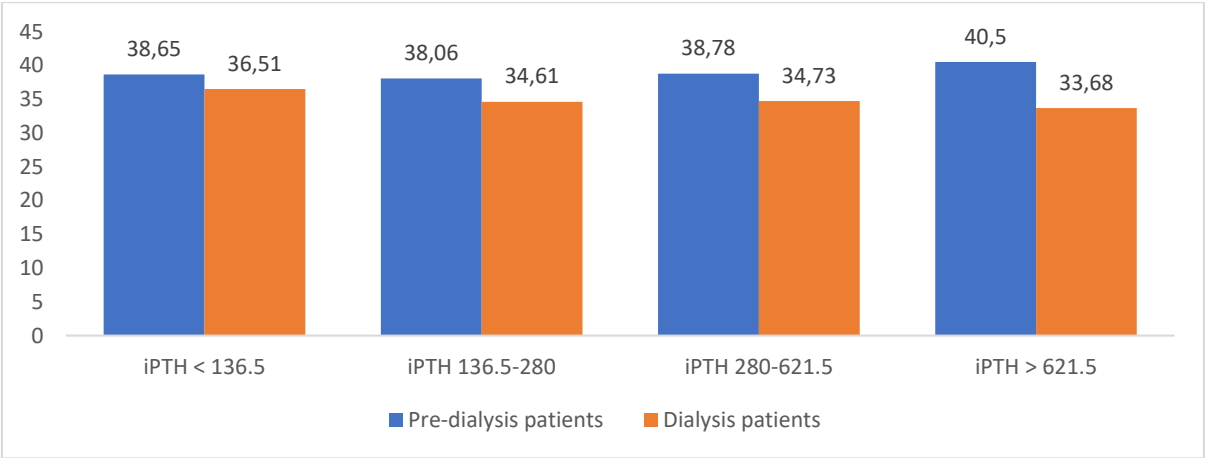

Fig. 11. Comparative analysis between iPTH and albumin according to the studied groups

4.3. Other factors responsible for the development of erythropoietin resistance (serum iron, folic acid, vitamin B12, soluble erythropoietin receptor, antibodies to erythropoietin, adequacy and duration of hemodialysis treatment) in patients with chronic kidney disease.

The mean ERI was 17.71 ± 6.37, with a minimum of 5.95 and a maximum of 41.0 (Fig. 12).

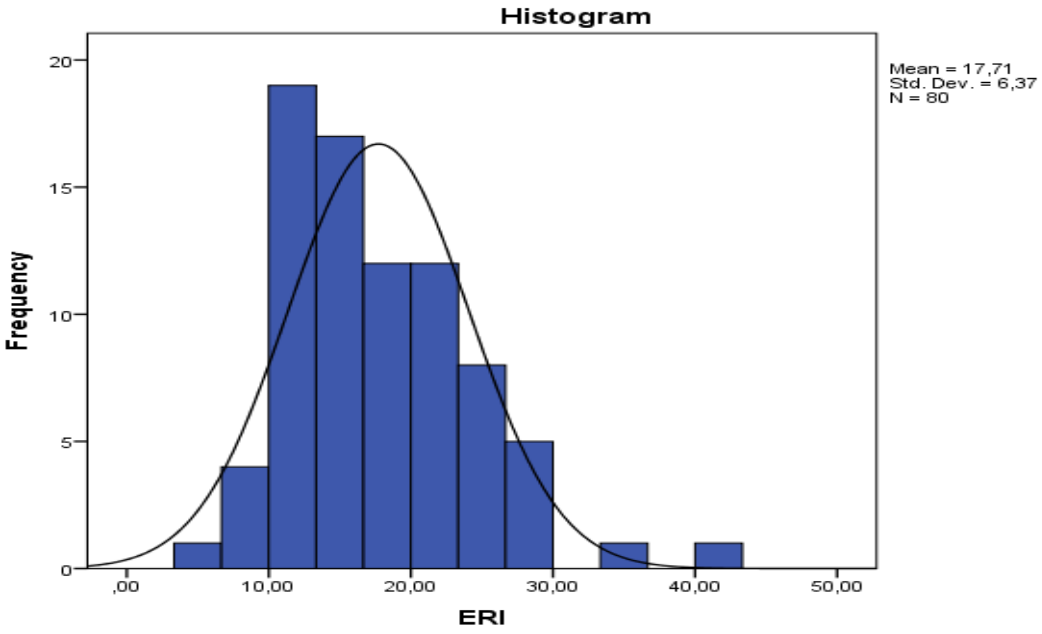

Fig. 12. Distribution according to ERI

There was a significant difference in the values of ERI according to the studied group (p = 0.003), and patients in the dialysis stage have a higher risk of developing erythropoietin resistance (Fig. 13).

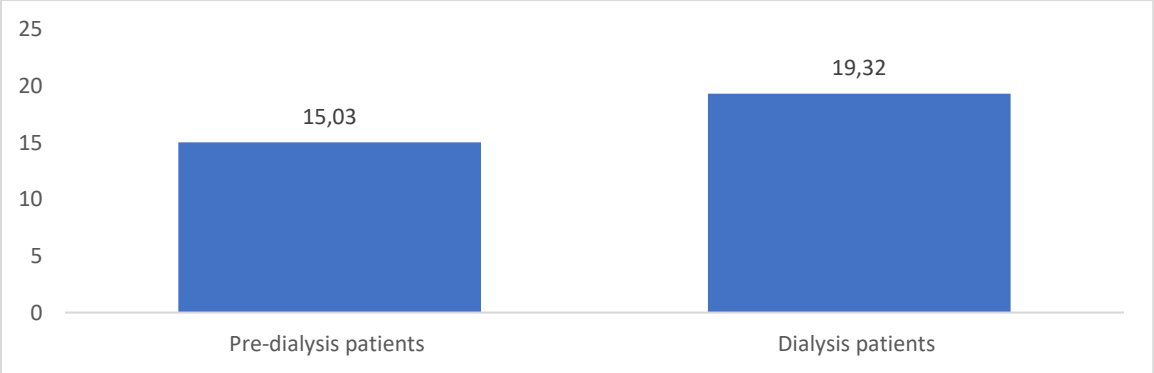

Fig. 13. Mean values of ERI according to the studied group

No relationship was found between ERI with gender and age of the studied patients. However, it can be said that in the pre-dialysis group ERI has lower values in men (p <0.05), while in the dialysis group there is no significant difference (Fig. 14).

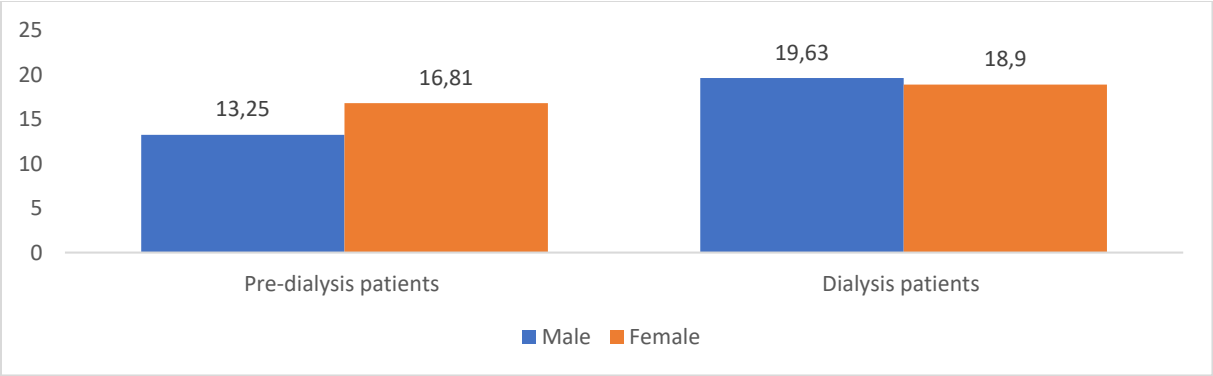

Fig. 14. Comparative analysis of ERI by gender and studied group

There was no significant difference in the mean values of ERI according to the diagnosis of the patients (Fig. 15).

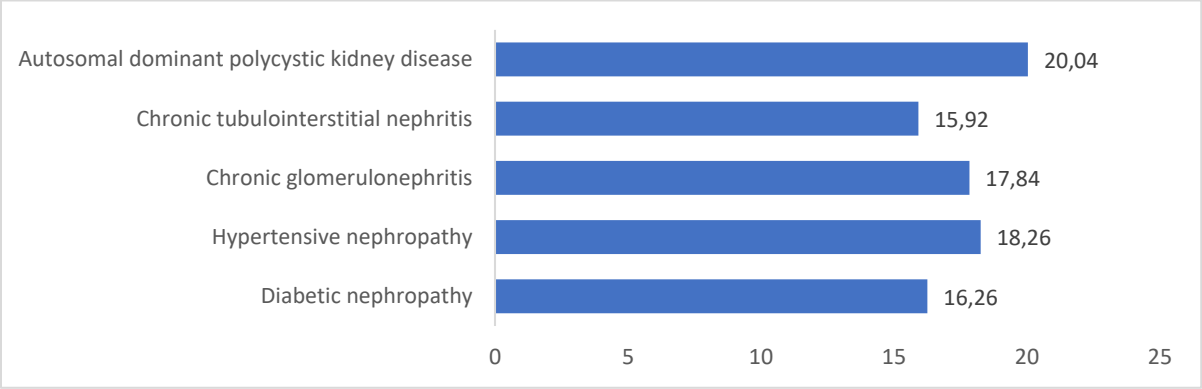

Fig. 15. Mean values of ERI according to the diagnosis

A significant difference was found in the mean concentrations of iPTH according to the ERI in the two studied groups (p = 0.042) (Fig. 16).

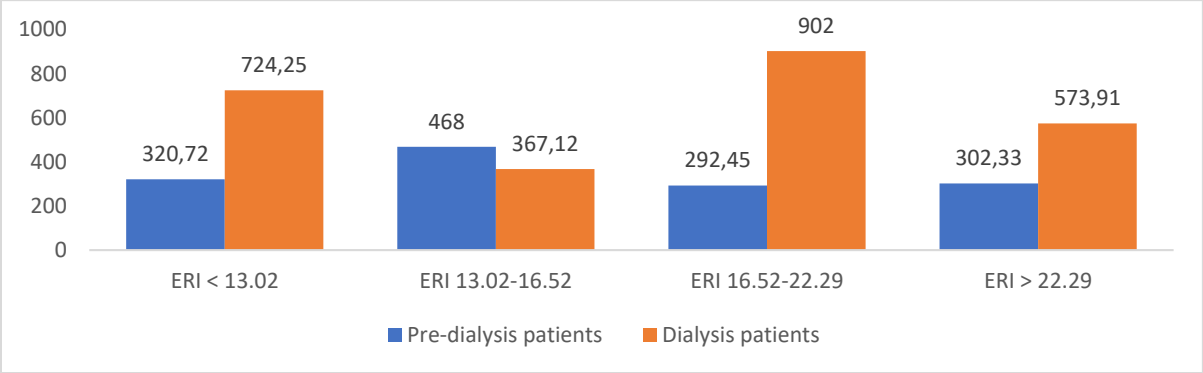

Fig. 16. Comparative analysis between ERI and iPTH according to the studied groups

No difference in ERI and calcium was found between patients in the pre-dialysis and dialysis groups. On the other hand, significant difference was found for ERI and phosphorus between the patients in both groups (p = 0.002) (Fig. 17).

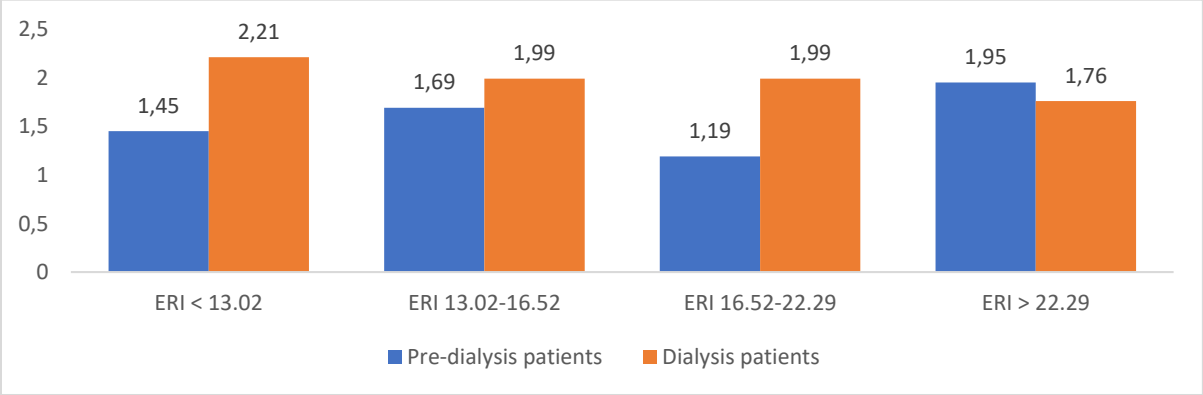

Fig. 17. Comparative analysis between ERI and phosphorus according to the studied groups

A directly proportional moderate relationship between ERI and iFGF-23 was found (r = 0.30; p = 0.02), with high ERI levels correlated with high iFGF-23 levels (Fig. 18).

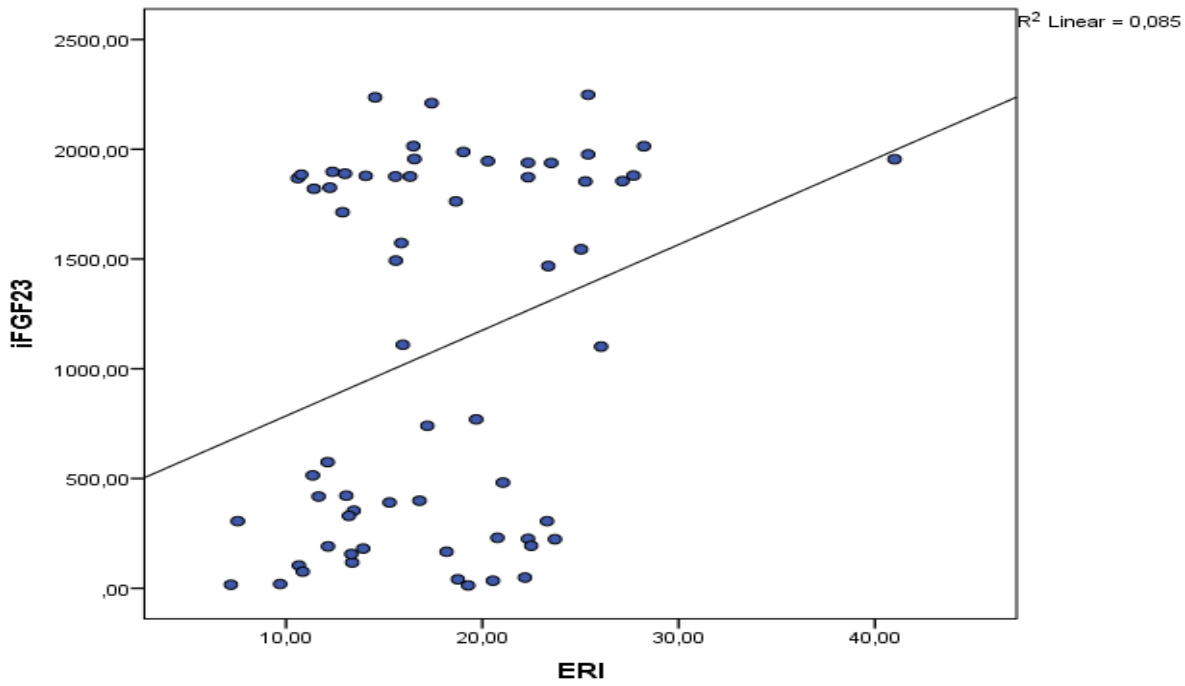

Fig. 18. Correlation between ERI and iFGF-23

A comparative analysis of iFGF-23 levels according to ERI revealed significantly higher values in dialysis patients (p <0.001), except for the highest ERI > 22.29 where in both groups the levels of iFGF-23 are high (respectively 1483.22pg/ml for patients in the pre-dialysis group to 1438.60pg/ml for patients in the dialysis group) (Fig. 19).

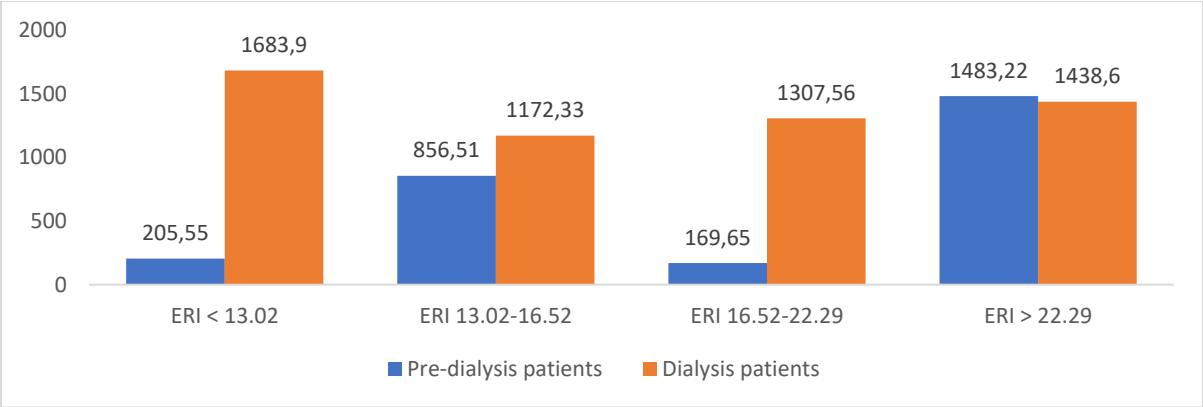

Fig. 19. Comparative analysis between ERI and iFGF-23 according to the studied groups

ERI correlates strongly negatively with hemoglobin levels (r = -0.588; p <0.001) (Fig. 20). No significant difference was found between hemoglobin levels according to ERI in the two studied groups.

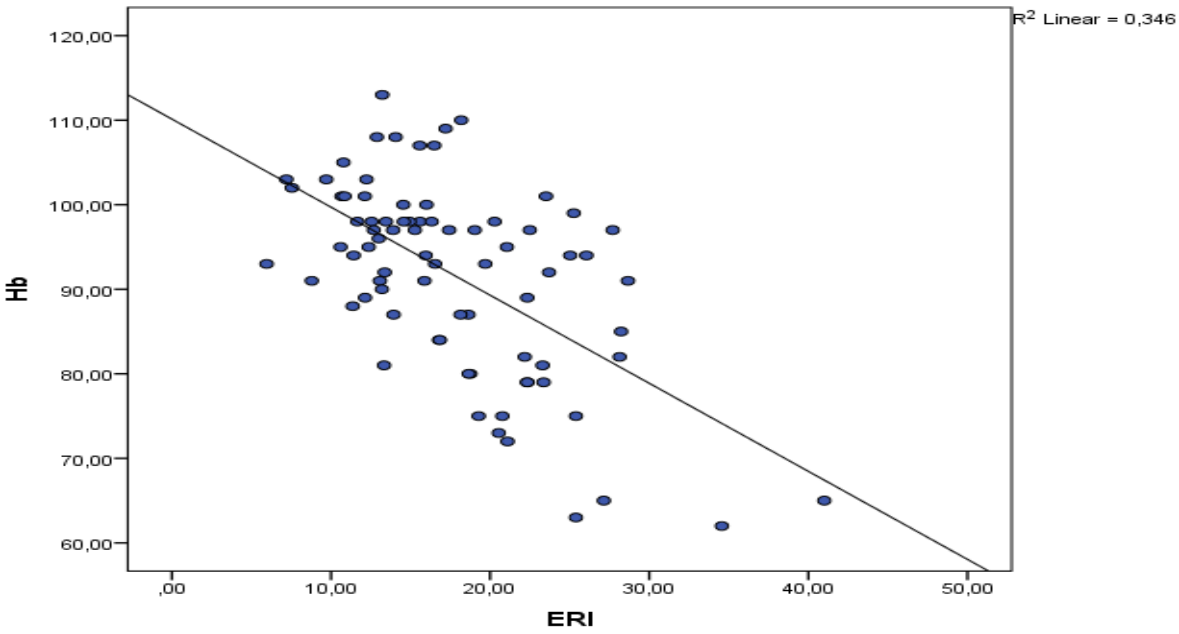

Fig. 20. Correlation analysis between ERI and hemoglobin

ERI correlates moderately inversely with URR (r = -0.305; p = 0.031), which means that inadequate dialysis treatment is associated with an increased risk of developing erythropoietin resistance.

Iron deficiency was also associated with an increased erythropoietin resistance index (r = -0.398; p = 0.004), with serum iron levels significantly lower at ERI> 15.0 (p <0.05) (Fig. 21).

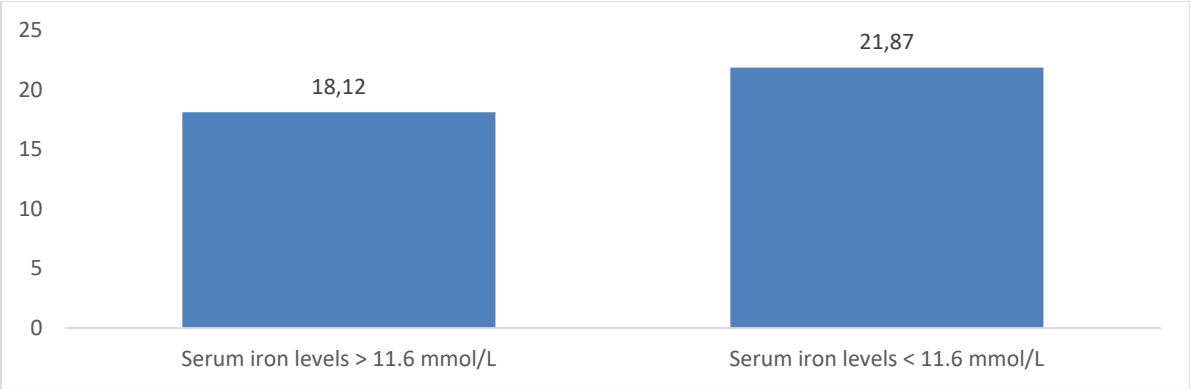

Fig. 21. Mean ERI values according to serum iron levels

In the analysis of TIBC according to the ERI and the studied groups, significantly higher levels of TIBC for ERI> 22.29 were observed in the dialysis group (p = 0.004) (Fig. 22).

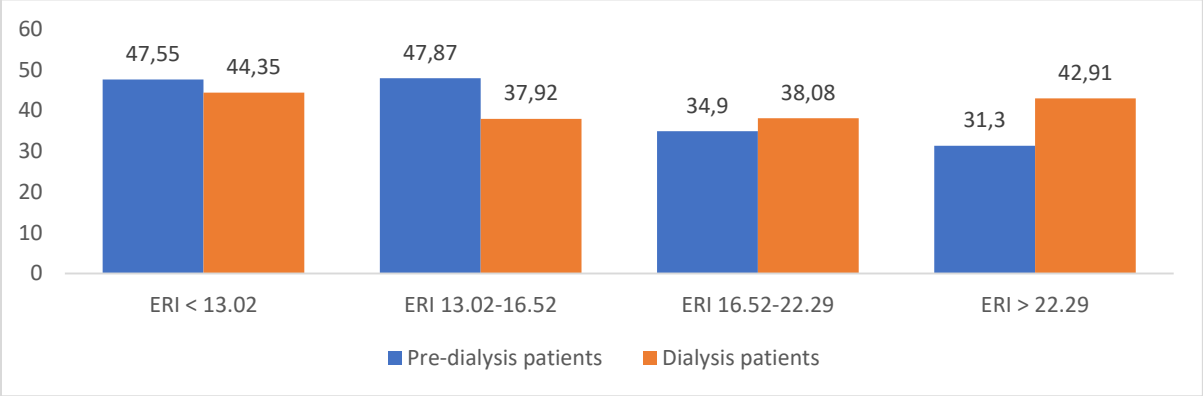

Fig. 22. Comparative analysis between ERI and TIBC according to the studied groups

TSAT also correlated negatively with ERI (r = -0.366; p = 0.009), with low TSAT values associated with the development of erythropoietin resistance. Patients with TSAT <20% had higher ERI levels than those with TSAT> 20% (p <0.05) (Fig. 23).

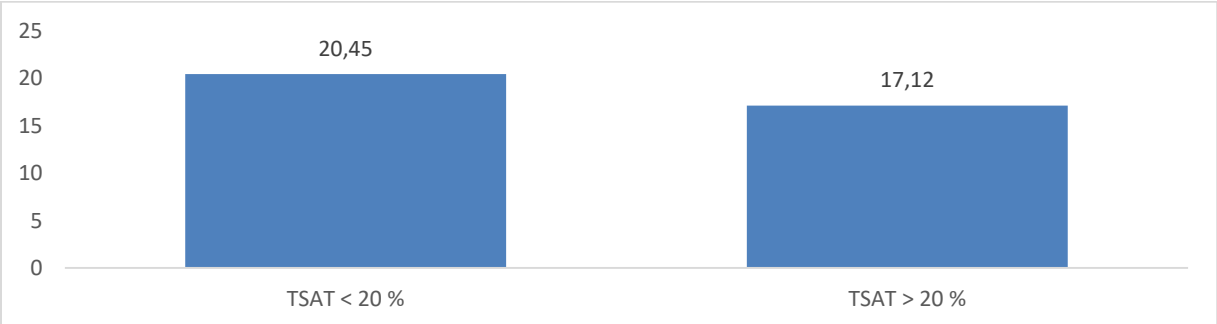

Fig. 23. Mean values of ERI according to TSAT

In a multi-regression step analysis, serum iron levels (p = 0.025) were found to be a major predictor of a hyporesponsiveness to ESA treatment.

The duration of hemodialysis treatment is moderately proportional to the ERI (r = 0.373; p = 0.008). The longer the hemodialysis treatment, the greater the risk of developing erythropoietin resistance (Fig. 24).

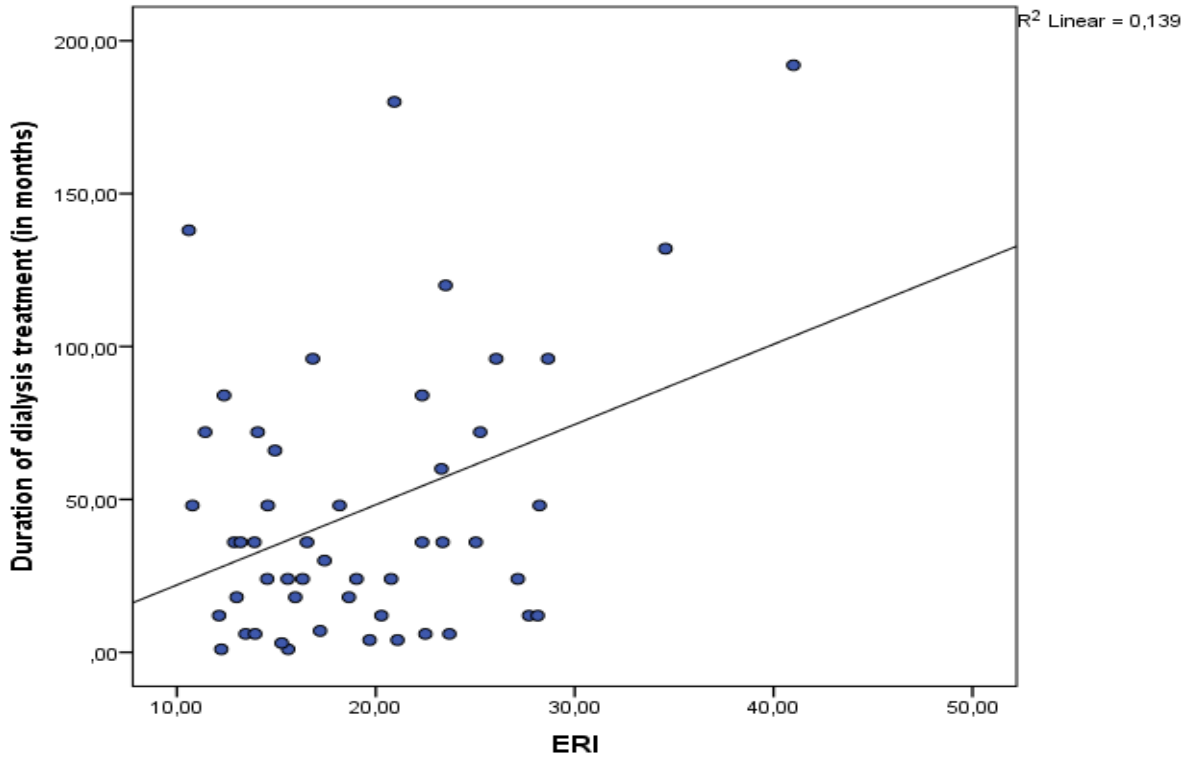

Fig. 24. Correlation between ERI and duration of dialysis treatment

No dependence or significant difference in folic acid and ERI was observed, with higher levels of folic acid in dialysis patients due to oral substitution (Fig. 25).

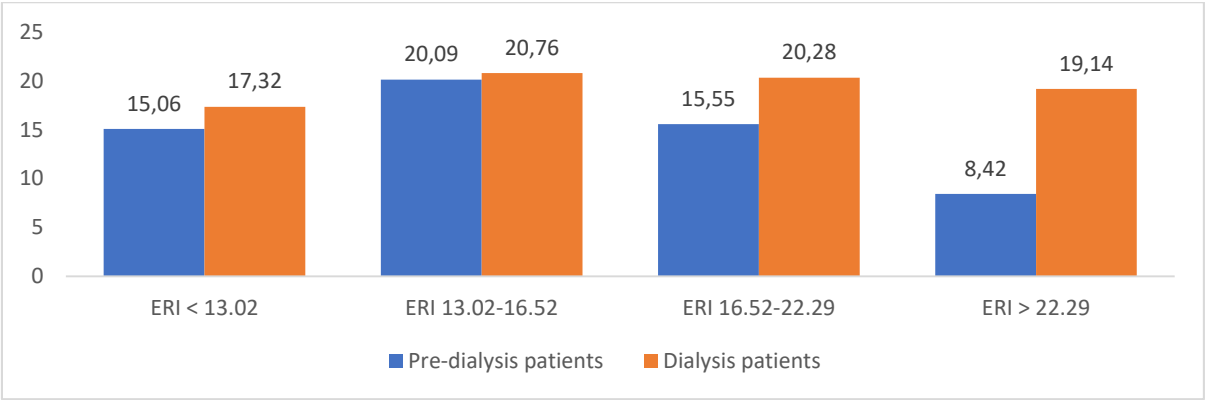

Fig. 25. Comparative analysis between ERI and folic acid according to the studied groups

Similar results were found for vitamin B12 concentrations and ERI in the two studied groups, where compensatory effects of Vitamin B12 treatment were observed in dialysis patients (Fig. 26).

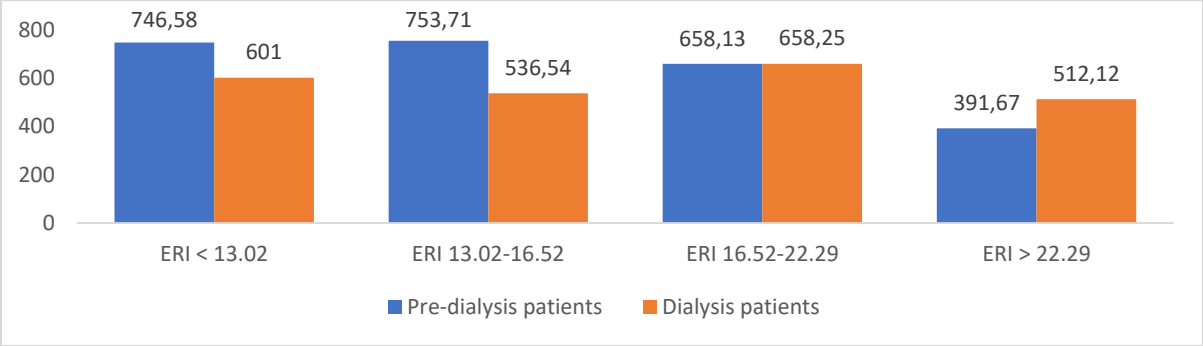

Fig. 26. Comparative analysis between ERI and vitamin B12 according to the studied groups

A significant difference in sEPOR concentrations was found according to ERI between the two studied groups (p = 0.022) (Fig. 27).

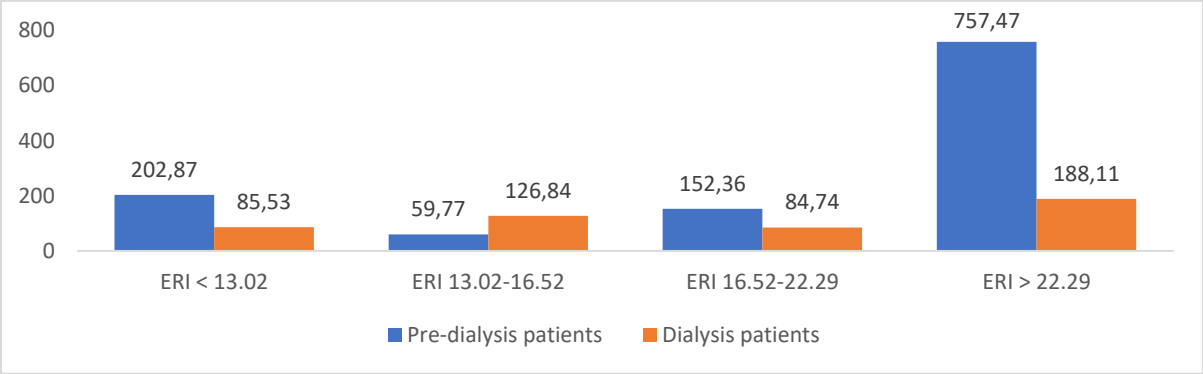

Fig. 27. Comparative analysis between ERI and sEPOR according to the studied groups

Although differences in erythropoietin antibody values were observed according to ERI between pre-dialysis and dialysis patients, no significant reliability was demonstrated (Fig. 28).

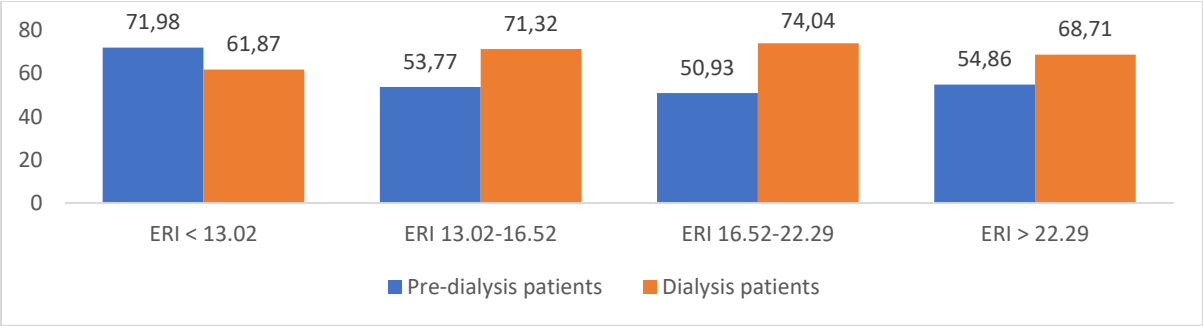

Fig. 28. Comparative analysis between ERI and antiEPOab according to the studied groups

4.4. Correlation between some markers of mineral and bone metabolism (including iPTH, FGF-23) and indicators of erythropoietin resistance (serum iron, folic acid, vitamin B12, soluble erythropoietin receptor, antibodies to erythropoietin, CRP) in patients with chronic kidney disease.

On the table 3 are presented the characteristics of the patients and a comparative analysis of the studied indicators between the patients in the pre-dialysis and dialysis stages.

Table 3. Characteristics of the patients according to the studied indicators

Indicator		Pre-dialysis patients (n=30)	Dialysis patients (n=50)	P value
BMI	mean±SD (range)	26.67±3.67 (21-35)	18.09±1.14 (16-21)	<0.001
CRP	mean±SD (range)	17.93±18.48 (0.13-81.19)	26.67±13.16 (1.22-57.06)	0.016
Folic acid	mean±SD (range)	15.70±8.59 (2.09-30.0)	19.54±8.71 (2.10-30.0)	0.05
Vitamin B12	mean±SD (range)	689.17±633.13 (210.0-3542.0)	567.76±254.15 (226.0-1975.0)	0.231
Serum Iron	mean±SD (range)	10.52±4.66 (1.10-23.60)	9.88±5.46 (1.20-30.10)	0.593
TIBC	mean±SD (range)	42.63±9.74 (19.30-58.70)	40.68±9.77 (20.90-64.20)	0.391
TSAT	mean±SD (range)	24.66±10.14 (12.0-59.0)	28.52±16.65 (2.30-95.0)	0.254
sEPOR	mean±SD (range)	211.47±378.85 (2.60-1676.5)	130.96±157.92 (35.4-970.80)	0.188
antiEPOab	mean±SD (range)	60.41±18.89 (25.11-98.42)	69.57±29.69 (27.46-132.47)	0.134

A weak inverse relationship was found between the levels of iPTH and BMI ($r = -0.211$; $p = 0.041$) (Fig. 29). A significant difference in the levels of iPTH and BMI between

the studied groups was found (p <0.001). Patients in the dialysis stage are mostly underweight (Fig. 30).

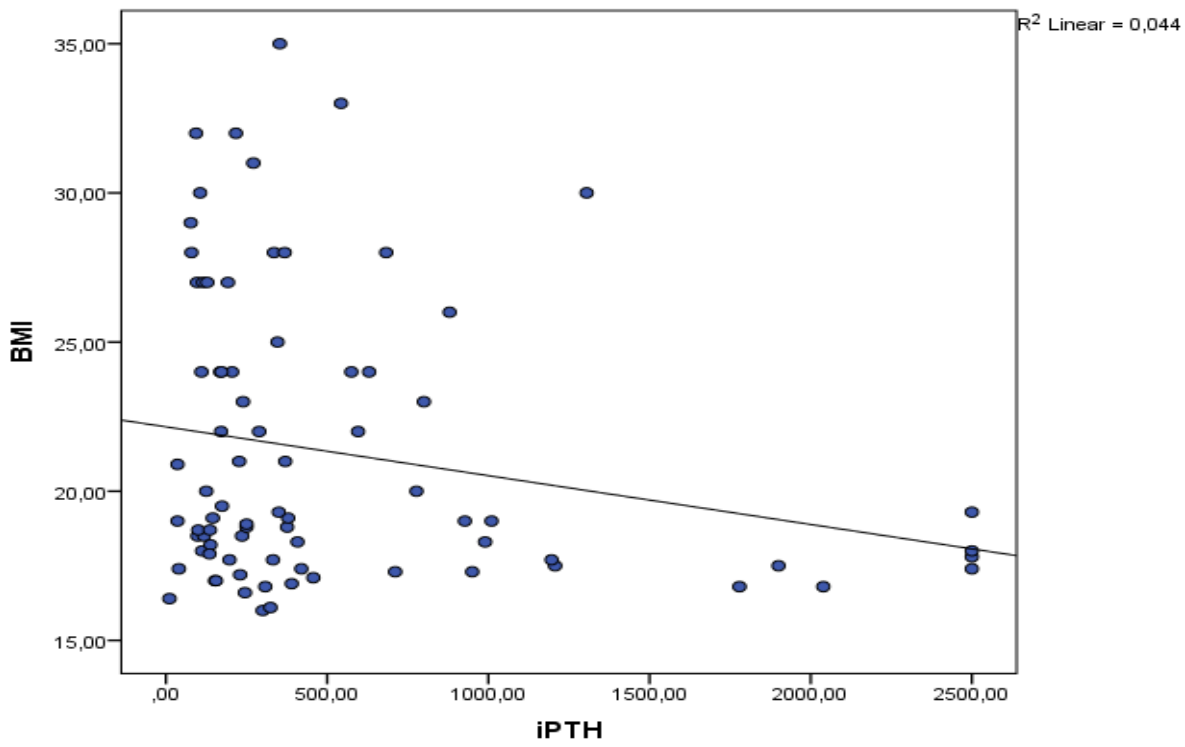

Fig. 29. Dependence between iPTH and BMI

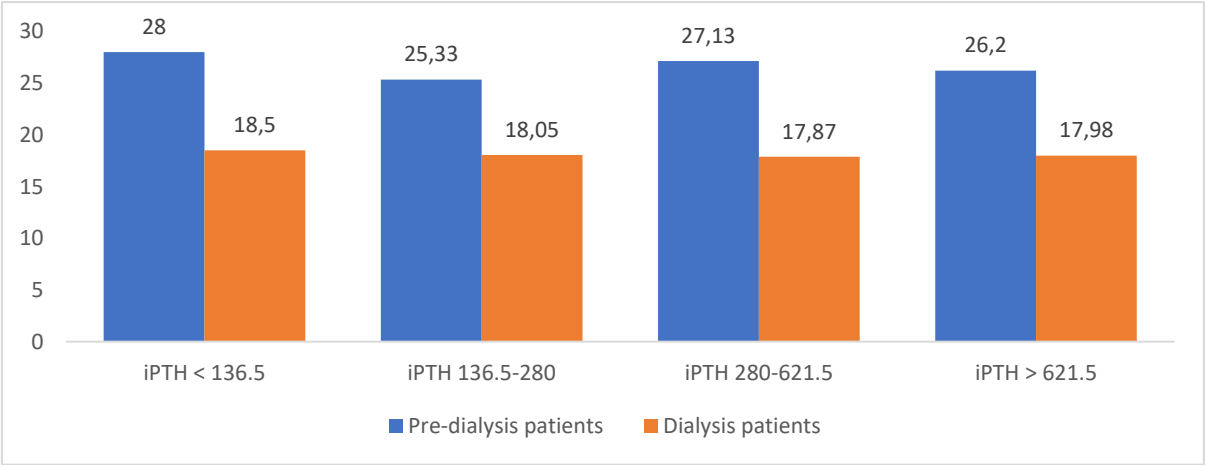

Fig. 30. Comparative analysis between iPTH and BMI levels according to the studied groups

The CRP analysis did not show an association with iPTH levels, but a significant difference was found between the study groups (p = 0.016). Patients in the dialysis group have significantly higher CRP levels (Fig.31).

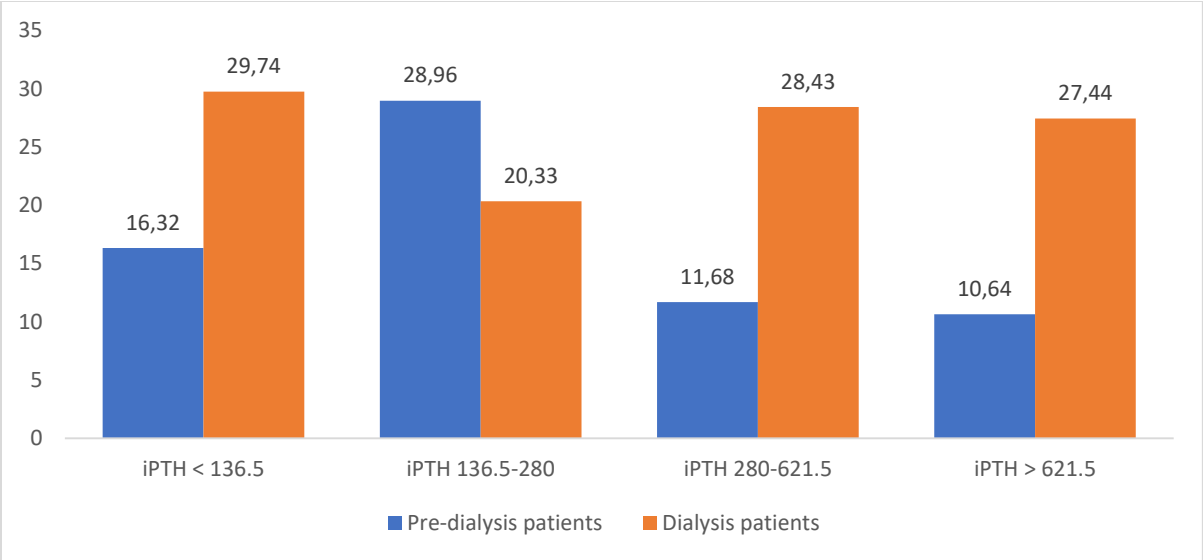

Fig. 31. Comparative analysis between iPTH and CRP according to the studied groups

There was a moderate proportional relationship between iPTH and folic acid (r = 0.313; p = 0.005) (Fig. 32), with a difference in the correlation coefficient between the two indicators in the pre-dialysis and dialysis groups (Fig. 33). A probable explanation for this fact is that the patients in the dialysis group undergo folic acid substitution therapy.

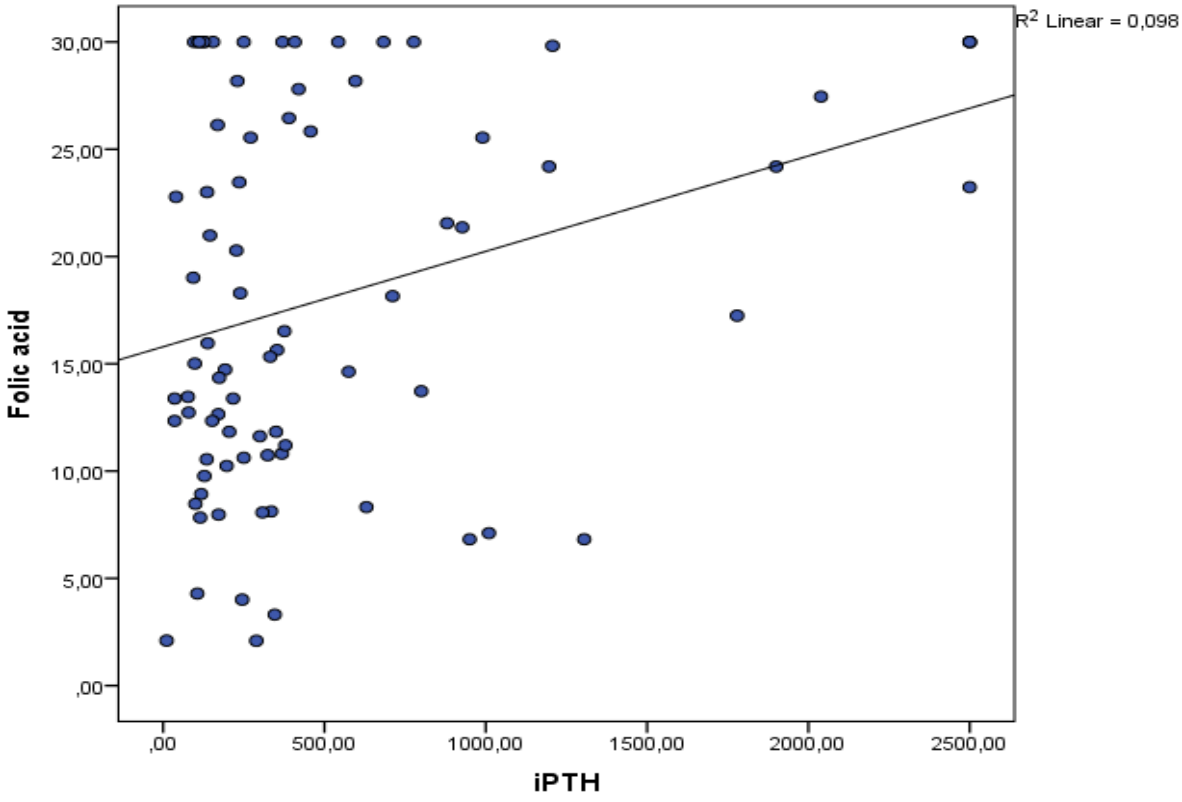

Fig. 32. Relationship between iPTH and folic acid

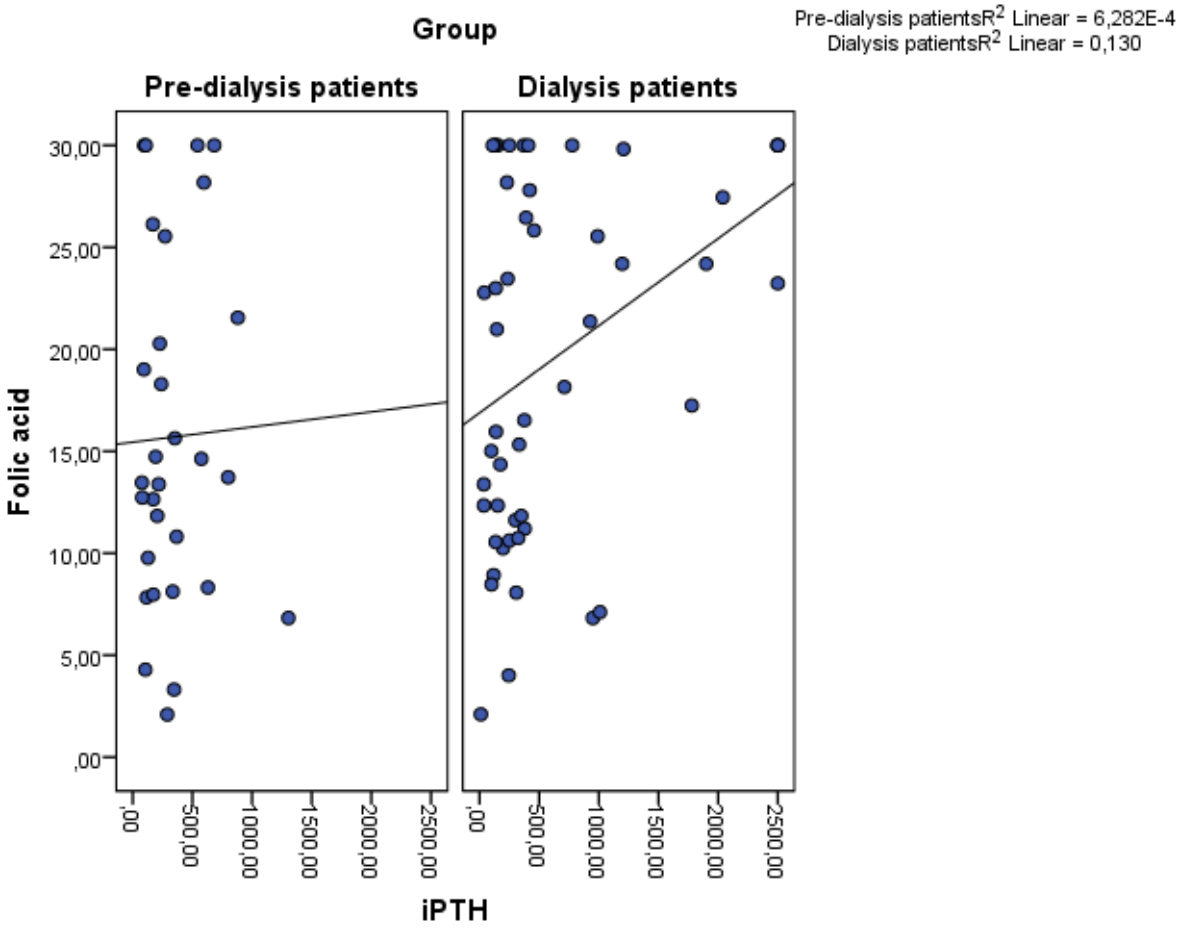

Fig. 33. Relationship between iPTH and folic acid in the two studied groups

Patients in the pre-dialysis group had approximately the same folic acid levels in the four quartiles of iPTH, while those in the dialysis group had folic acid levels in iPTH <136.5pg/ml significantly lower than those in the iPTH group> 621.5pg / ml (17.21 to 23.01, respectively; p <0.05) (Fig. 34).

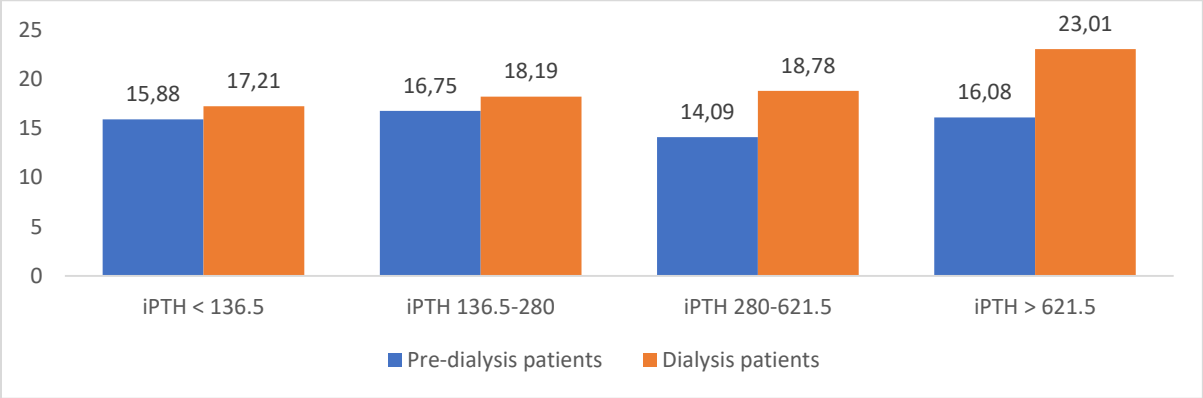

Fig. 34. Comparative analysis between iPTH and folic acid according to the studied groups

No relationship was found between the levels of iPTH and vitamin B12, serum iron, TIBC, TSAT, sEPOR, antiEROab and no significant difference between the two studied groups. Dependence was not found with URR.

An inversely strong relationship was found between iFGF-23 and BMI (r = -0.503; p <0.001) (Fig. 35).

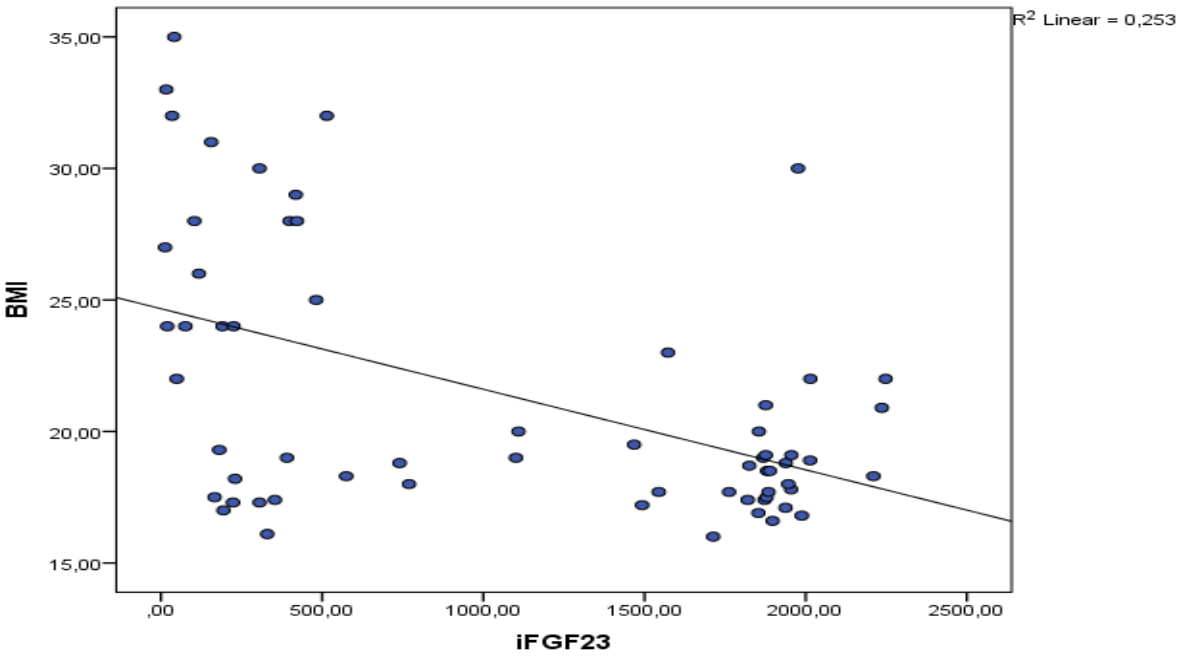

Fig. 35. Relationship between iFGF-23 and BMI levels

No correlation was found between iFGF-23 levels with CRP, folic acid, vitamin B12, serum iron, TIBC, TSAT, sEPOR, erythropoietin antibodies and URR.

There was a significant difference in the levels of iFGF-23 and CRP according to the studied groups (p <0.05), as in patients in the pre-dialysis group there was a decrease in CRP levels in iFGF-23 <1880.14 pg/ml, while in patients in the dialysis group we observed an increase in CRP levels at iFGF-23 <1109.19 pg/ml, followed by a slow decrease in CRP values that remain above the reference limit (Fig. 36).

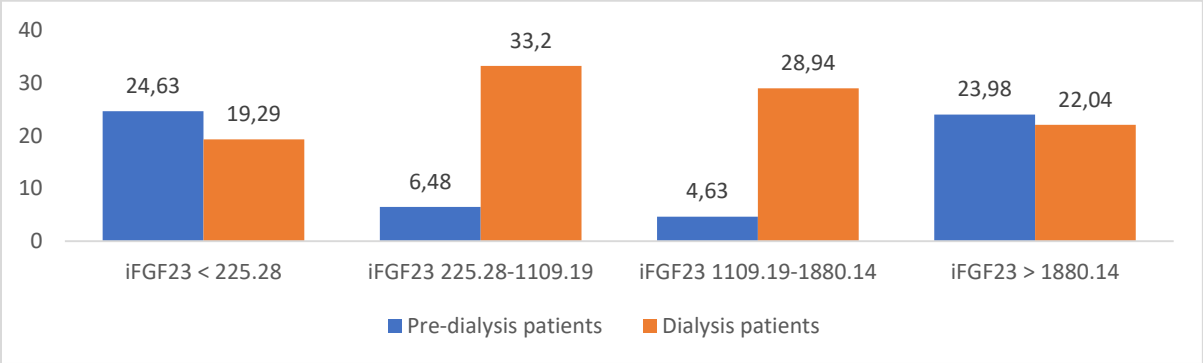

Fig. 36. Comparative analysis between iFGF-23 and CRP according to the studied groups

In the analysis of changes in folic acid levels according to iFGF-23 levels, there was a statistically significant difference between the two studied groups (p <0.05), as folic acid levels in dialysis patients were higher than in pre-dialysis patients, which is due to the treatment in the first group (Fig. 37).

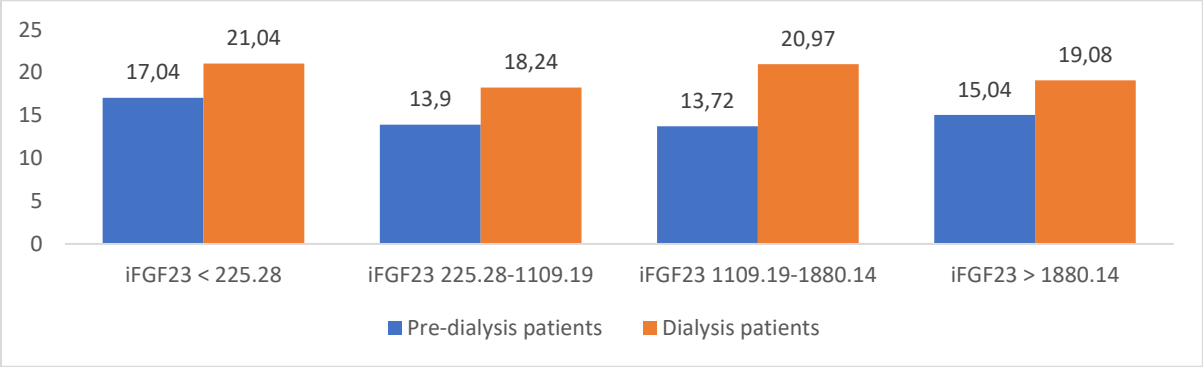

Fig. 37. Comparative analysis between iFGF-23 and folic acid according to the studied groups

In Fig. 38 is presented a comparative analysis between the levels of iFGF-23 and Vitamin B12 according to the studied groups, where there are two significant differences. The first difference is in the levels of Vitamin B12, which significantly decrease with increasing concentrations of iFGF-23 in patients in the pre-dialysis group (p <0.01). The second difference was in the levels of Vitamin B12 in the two studied groups, as with iFGF-23 from 1109.19 - 1880.14 pg/ml and >1880.14 pg/ml, the concentrations of Vitamin B12 were significantly higher in patients in the dialysis stage (p <0.01).

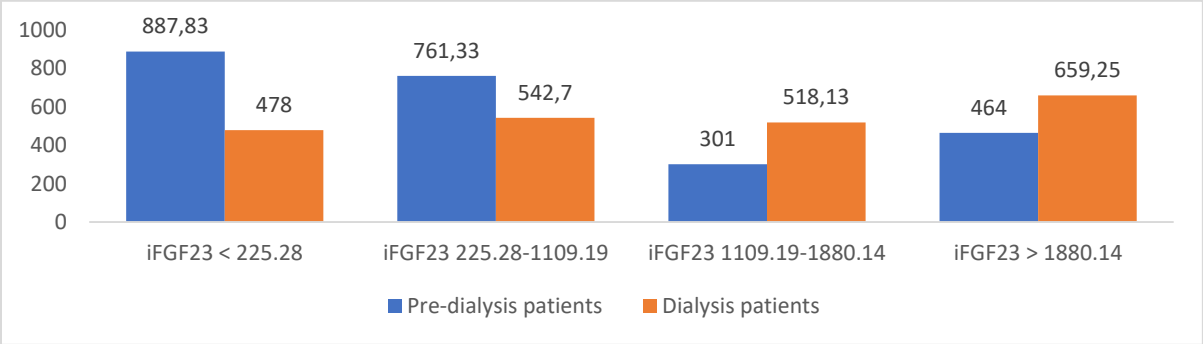

Fig. 38. Comparative analysis between the levels of iFGF-23 and Vitamin B12 according to the studied groups

In the analysis of iFGF-23 and sEPOR levels according to the studied groups, there was a significant difference in iFGF23 <225.28 pg/ml, where sEPOR values were significantly higher in patients in the dialysis group (p <0.001) (Fig. 39). The other significant change was

at iFGF-23> 1880.14 pg/ml, where sEPOR values were significantly higher in patients in the pre-dialysis group (p <0.001).

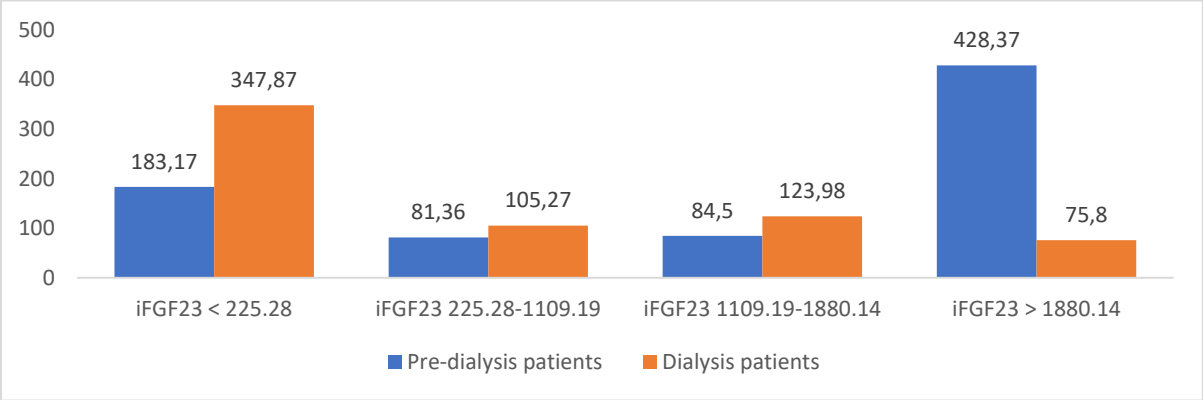

Fig. 39. Comparative analysis between iFGF-23 and sEPOR according to the studied groups

Significant differences in antiEPOab concentrations were observed only at iFGF-23 <225.28 pg/ml, where antiEPOab levels were significantly higher in dialysis patients (p <0.001) (Fig. 40).

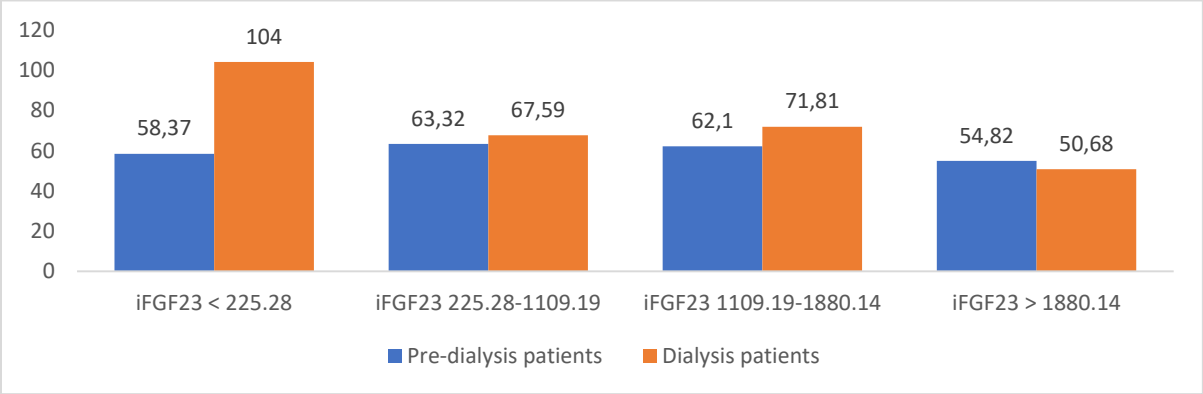

Fig. 40. Comparative analysis between iFGF-23 and antiEPOab according to the studied groups

4.5. Comparison of the results of the treatment of secondary hyperparathyroidism and anemia and the required doses of drugs

There was a statistically significant difference in the treatment of secondary hyperparathyroidism in patients in the pre-dialysis and dialysis stages (p = 0.001) (Fig. 41). Patients in the pre-dialysis group were treated mainly with calcitriol 0.25 mcg/day and alfacalcidol 1 mcg/day.

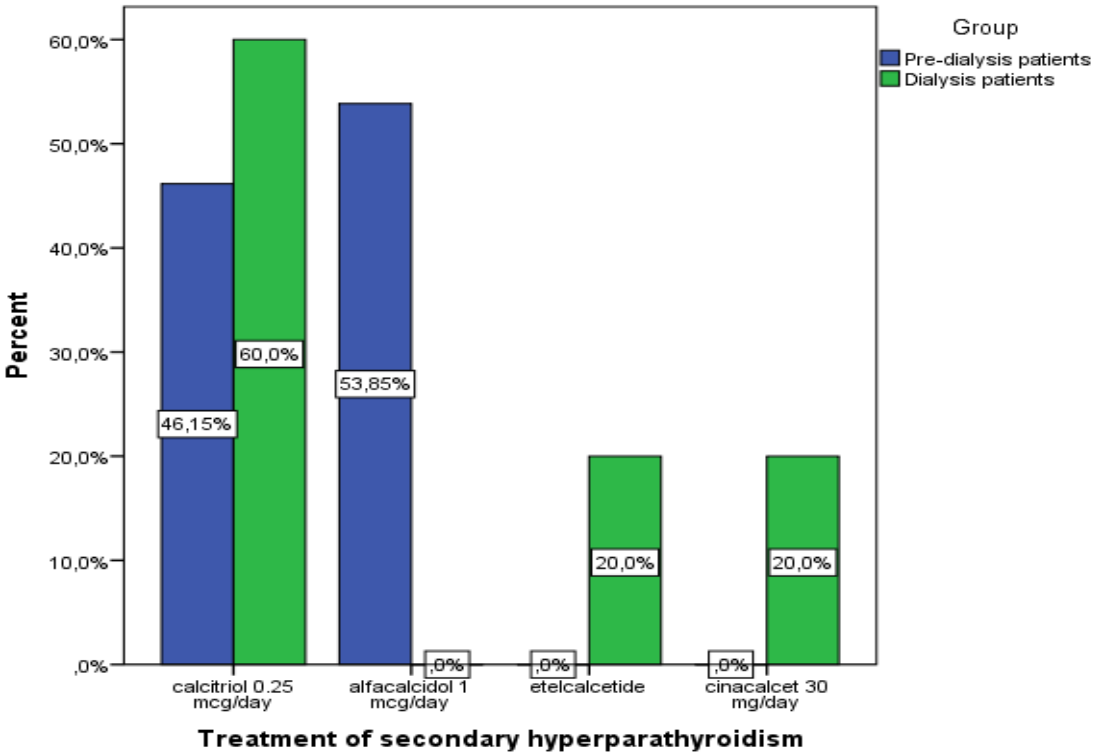

Fig. 41. Comparative analysis of the treatment of secondary hyperparathyroidism in patients in the pre-dialysis and dialysis stages of CKD

A significant difference ($p < 0.001$) and significant dependence were found with respect to iron therapy in the two groups ($r = 0.850$; $p < 0.001$) (Fig. 42). Patients in the dialysis group are treated mainly with 100 mg of iron sucrose at different time intervals.

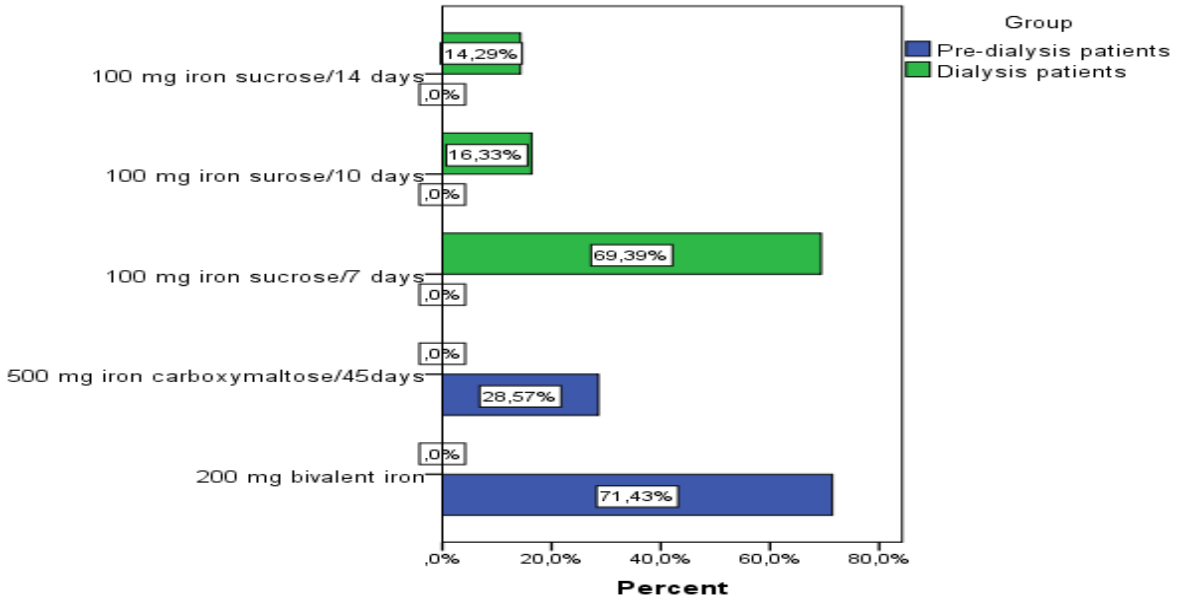

Fig.42. Comparative analysis of iron therapy in patients in pre-dialysis and dialysis stages of CKD

There was a significant difference in the average dose of ESA in patients from the two studied groups (p <0.001), and in those in the dialysis group there was a need for higher doses of ESA (Fig. 43).

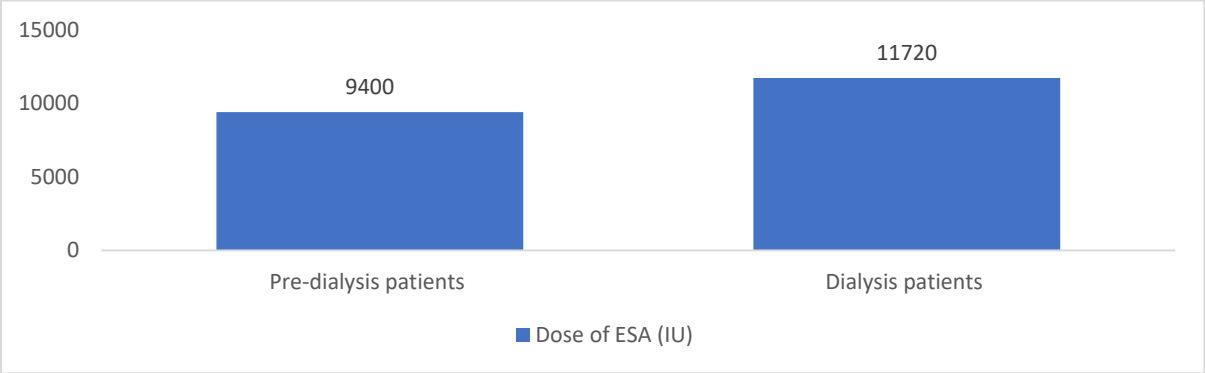

Fig. 43. Mean dose of ESA (IU) in patients in both studied groups

There was a statistically significant difference in the mean doses of ESA according to the use of iron supplements (p = 0.007), with patients receiving 200 mg of ferrous iron treated with the lowest doses of ESA (9333.33 IU), while those receiving 100 mg iron sucrose/7 days are treated with the highest doses of ESA (11911.76IU) (Fig. 44).

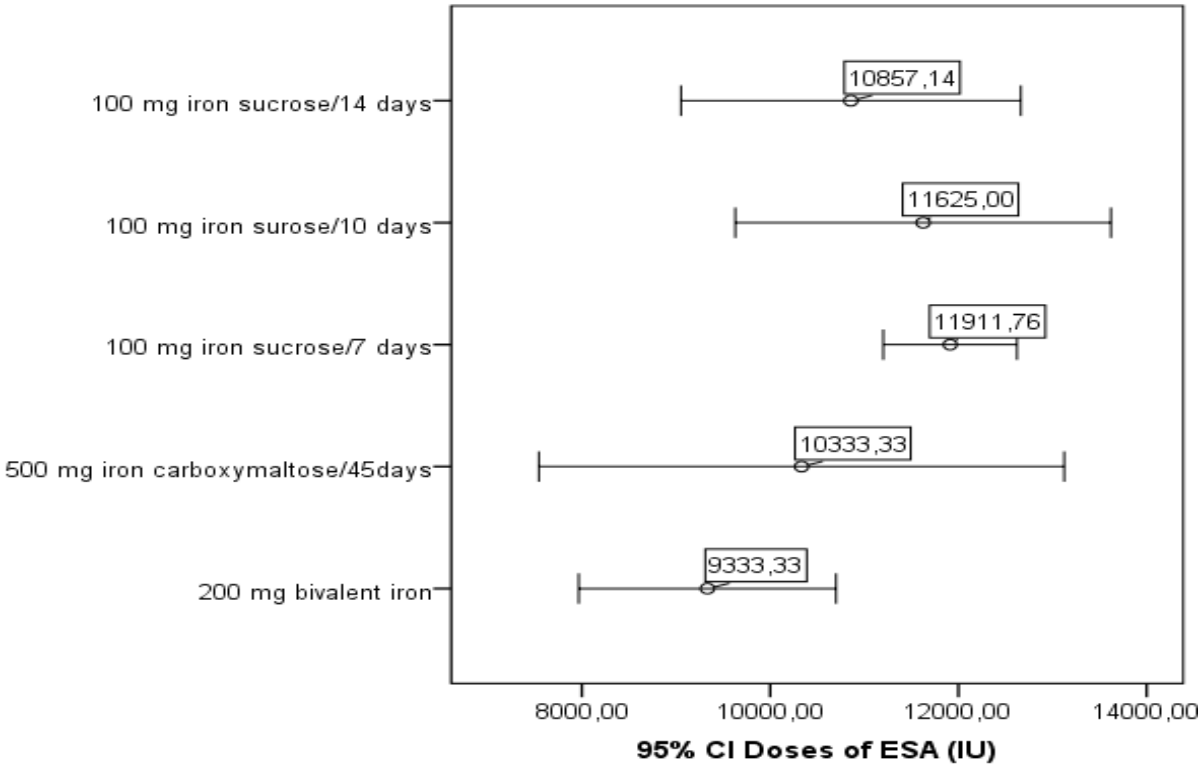

Fig. 44. Comparative analysis of average doses of ESA (IU) according to the intake of individual iron preparations

In the comparative analysis of ERI according to the conducted therapy of secondary hyperparathyroidism, a significant difference was observed (p = 0.002), as patients taking cinacalcet 30 mg/day had the highest ERI values (Table 4).

Table 4. Comparative analysis of ERI values according to the therapy of secondary hyperparathyroidism

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Without treatment	47	17,6553	5,09059	16,1606	19,1499	8,79	28,23
0,25 mcg Calcitriol/day	18	16,7661	6,99418	13,2880	20,2442	5,95	34,56
1mcg Alfacalcidol/day	7	15,4357	6,20085	9,7009	21,1705	7,19	22,33
Etelcalcetide	4	14,8975	5,92841	5,4641	24,3309	10,60	23,51
Cinacalcet 30mg/day	4	29,4325	8,40539	16,0576	42,8074	20,93	41,00
Total	80	17,7120	6,36988	16,2944	19,1295	5,95	41,00

Patients who were without treatment or taking calcitriol 0.25 mcg/day had the lowest levels of iPTH (387.28 pg/ml and 375.88 pg/ml, respectively), while those treated with etelcalcetide and cinacalcet 30 mg/day had the highest levels of iPTH (1272.75pg/ml and 1954.00pg ml, respectively) (p <0.001) (Table 5).

Table 5. Comparative analysis of iPTH according to the therapy of secondary hyperparathyroidism

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Without treatment	47	387,2830	458,80151	252,5738	521,9921	35,60	2500,00
0,25 mcg Calcitriol/day	18	375,8844	476,43184	138,9605	612,8084	10,90	1779,00
1mcg Alfacalcidol/day	7	520,4286	378,04403	170,7962	870,0610	217,00	1305,00
Etelcalcetide	4	1272,7500	874,72982	-119,1403	2664,6403	457,00	2500,00
Cinacalcet 30mg/day	4	1954,0000	814,20432	658,4192	3249,5808	777,00	2500,00
Total	80	518,9778	620,09641	380,9821	656,9734	10,90	2500,00

A significant difference was also found in iFGF-23 levels according to the treatment of secondary hyperparathyroidism (p <0.001), with the lowest iFGF-23 levels observed in patients receiving 1 mcg alfacalcidol/day (174.13pg/ml), and the highest in patients treated with etelcalcetide and cinacalcet 30 mg/day (1876.06pg/ml and 1904.79pg/ml, respectively) (Table 6).

Table 6. Comparative analysis of iFGF-23 values according to the therapy of secondary hyperparathyroidism

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Without treatment	40	973,3476	781,61683	723,3745	1223,3208	12,88	2247,92
0,25 mcg Calcitriol/day	11	1564,0853	774,87343	1043,5182	2084,6523	75,72	2236,25
1mcg Alfacalcidol/day	6	174,1317	169,32084	-3,5597	351,8230	16,31	421,85
Etelcalcetide	4	1876,0575	48,13893	1799,4577	1952,6573	1820,10	1937,34
Cinacalcet 30mg/day	2	1904,7900	70,52683	1271,1316	2538,4484	1854,92	1954,66
Total	63	1087,2610	821,42672	880,3874	1294,1347	12,88	2247,92

IPTH levels differed significantly according to the treatment of anemia ($p < 0.05$), with the lowest iPTH values observed in patients treated with 100 mg iron sucrose /10 days (163.61pg/ml), followed by those treated with 500 mg iron carboxymaltose /45 days (189.00pg/ml). The highest concentrations of iPTH were found with 100 mg of iron sucrose / 7 days (746.45pg/ml) and 100 mg of iron sucrose /14 days (617.65pg/ml) (Table 7).

Table 7. Comparative analysis of iPTH levels according to the therapy of anemia

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Without treatment	10	333,7000	213,43490	181,0179	486,3821	115,00	800,00
200 mg bivalent iron	15	402,3467	359,33315	203,3546	601,3387	79,30	1305,00
500 mg iron carboxymaltose/45days	6	189,0000	109,61569	73,9654	304,0346	77,00	353,00
100 mg iron sucrose/7 days	34	746,4565	801,91169	466,6563	1026,2567	35,60	2500,00
100 mg iron surose/10 days	8	163,6125	95,68879	83,6147	243,6103	10,90	332,00
100 mg iron sucrose/14 days	7	617,6571	680,15745	-11,3836	1246,6979	35,60	1900,00
Total	80	518,9778	620,09641	380,9821	656,9734	10,90	2500,00

The results of the analysis of iFGF-23 levels according to the treatment of anemia show that there is a significant difference ($p = 0.002$), with the lowest values of iFGF-23 have patients treated with 500 mg of iron carboxymaltose / 45 days (440.92pg/ml), followed by those without treatment (469.95pg/ml). The highest concentrations of iFGF-23 were reported in patients treated with 100 mg of iron sucrose/10 days (1679.55pg/ml) (Table 8).

Table 8. Comparative analysis of iFGF-23 levels according to the therapy of the anemia

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Without treatment	6	469,9478	567,05938	-125,1445	1065,0402	48,89	1572,73
200 mg bivalent iron	10	592,9272	828,87646	-,0153	1185,8697	12,88	2247,92
500 mg iron carboxymaltose/45days	6	440,9240	767,11872	-364,1177	1245,9657	19,42	1976,47
100 mg iron sucrose/7 days	29	1346,1806	737,79588	1065,5380	1626,8231	180,90	2210,19
100 mg iron surose/10 days	6	1679,5500	309,87637	1354,3547	2004,7453	1109,19	1897,09
100 mg iron sucrose/14 days	6	1331,0673	864,47313	423,8584	2238,2762	166,44	2236,25
Total	63	1087,2610	821,42672	880,3874	1294,1347	12,88	2247,92

Examination of the relationship between ERI values and ESA dose revealed a strong proportional relationship ($r = 0.551$; $p < 0.001$), which showed that with the increasing of ESA doses increases an ERI value (Fig. 45).

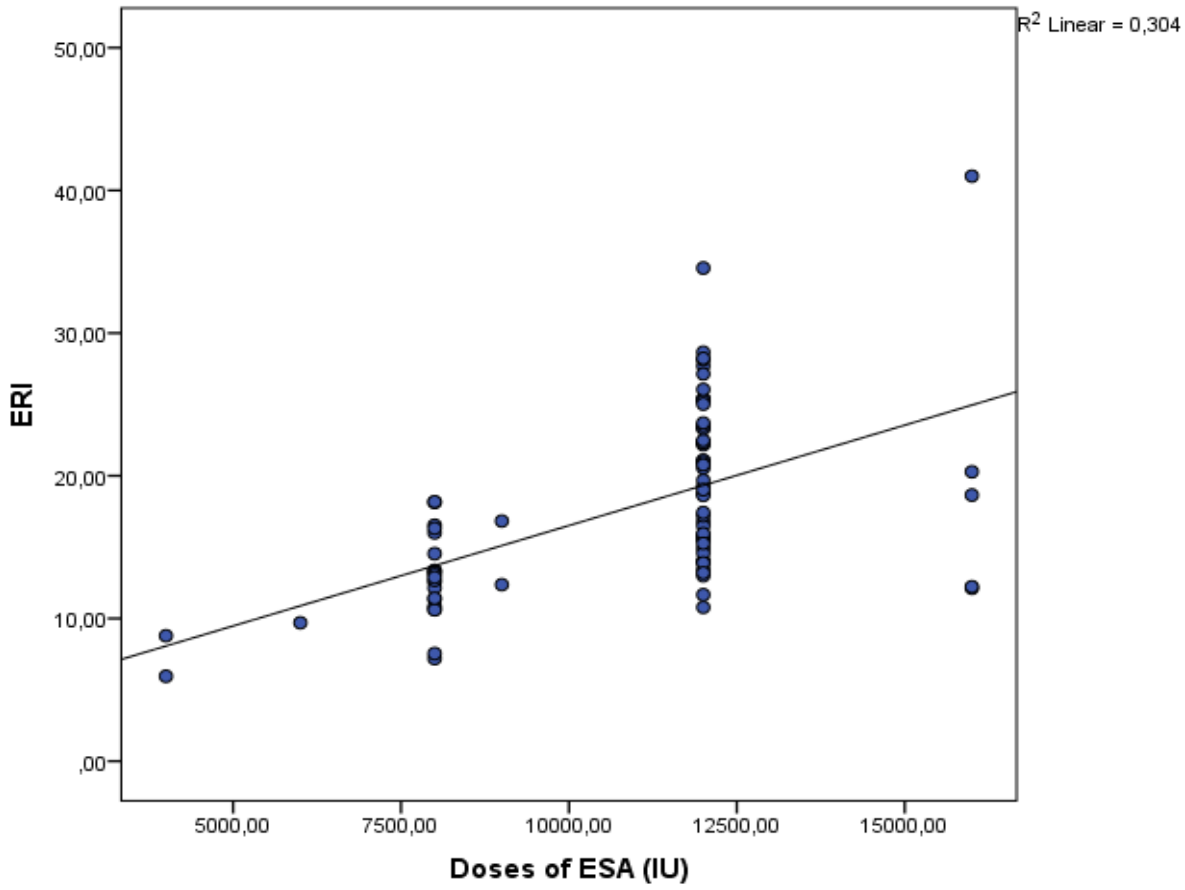

FIG. 45. Correlation analysis between ERI and ESA doses

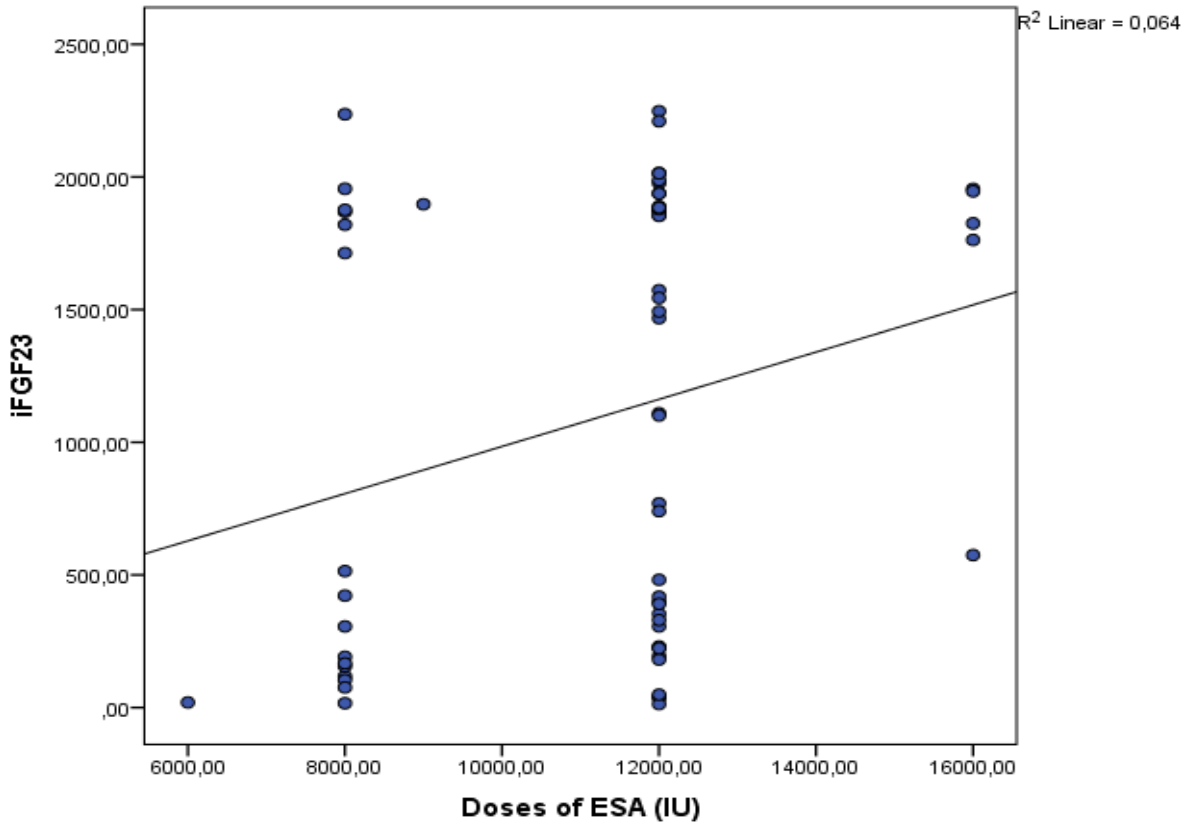

FIG. 46. Correlation analysis between FGF-23 and ESA doses

A proportionally weak dependence was also found for iFGF-23 levels according to ESA doses (r = 0.252; p = 0.046) (Fig. 46). According to the results, it can be said that in 6.4% of cases of elevated iFGF-23 values, high doses of ESA are important.

There is a tendency for patients in the dialysis stage who are on dialysis treatment to have a longer period of intake of 100 mg of iron sucrose (p <0.05) (Table 9).

Table 9. Comparative analysis between the duration of dialysis treatment and the treatment of anemia

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Without treatment	1	132,0000	.	.	.	132,00	132,00
200 mg bivalent iron	34	51,2647	49,63156	33,9474	68,5820	1,00	192,00
500 mg iron carboxymaltose/45days	8	30,7500	23,22406	11,3342	50,1658	12,00	84,00
100 mg iron sucrose/7 days	7	29,0000	27,48939	3,5766	54,4234	1,00	72,00
100 mg iron surose/10 days	50	46,4800	45,58206	33,5257	59,4343	1,00	192,00

In the comparative analysis of the duration of dialysis treatment according to the therapy of secondary hyperparathyroidism, a significant difference was observed, which shows that patients with the longest duration of dialysis treatment are treated with etelcalcetide (100.5 months) and cinacalcet 30 mg/day (123.00 months). ($p < 0.001$) (Table 10).

Table 10. Comparative analysis between the duration of dialysis treatment and the treatment of secondary hyperparathyroidism

	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Without treatment	30	32,6667	29,50550	21,6491	43,6842	1,00	96,00
0,25 mcg Calcitriol/day	12	37,5000	34,14541	15,8050	59,1950	12,00	132,00
1mcg Alfacalcidol/day	4	100,5000	33,71943	46,8449	154,1551	72,00	138,00
Etelcalcetide	4	123,0000	78,61298	-2,0908	248,0908	24,00	192,00
Cinacalcet 30mg/day	50	46,4800	45,58206	33,5257	59,4343	1,00	192,00

In the present study, no relationship was found between the duration of treatment and antiEPOab, as well as with respect to ESA doses. On the other hand, a significant difference was observed in sEPOR values and ESA doses ($p = 0.002$) (Fig. 47).

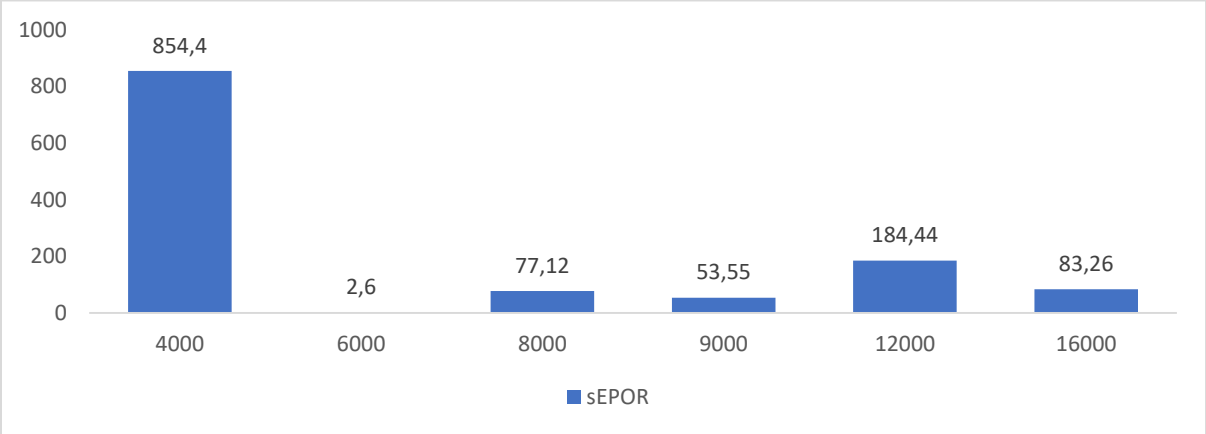

Fig. 47. Mean values of sEPOR according to ESA doses

An analysis of the relationship between Hb and sEPOR revealed an inversely weak relationship (r = -0.201; p = 0.036) (Fig. 48), which shows that patients with lower sEPOR values have higher Hb levels.

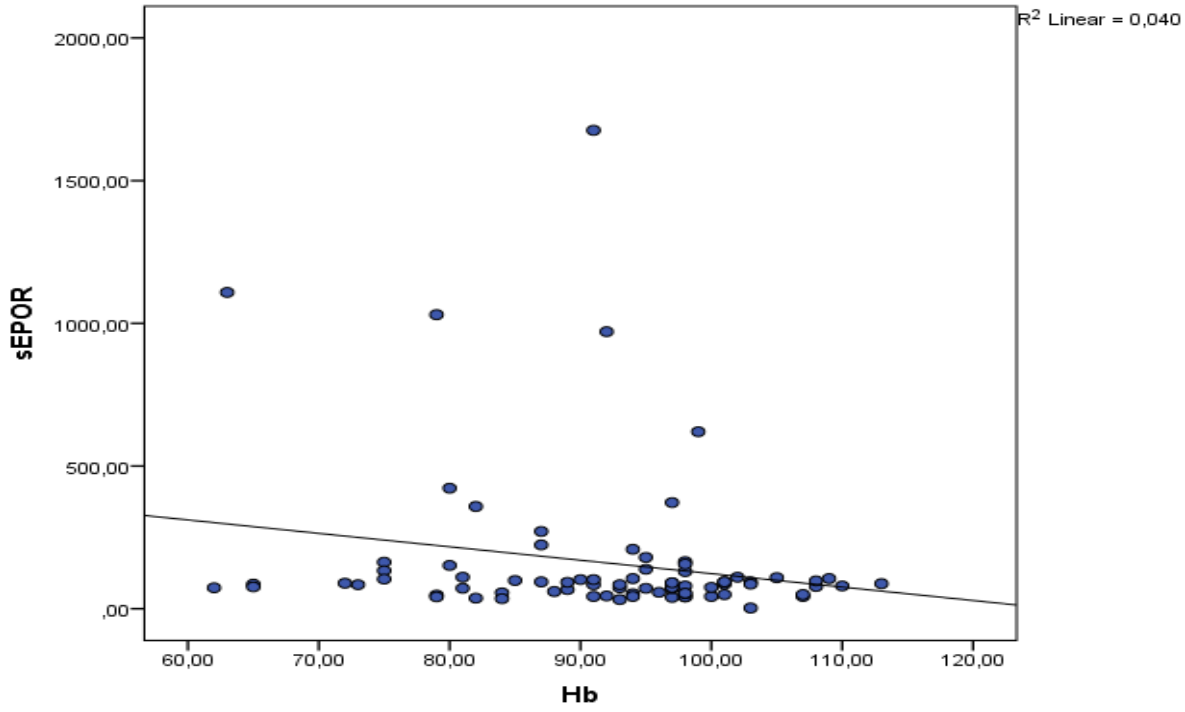

Fig. 48. Correlation analysis between Hb and sEPOR

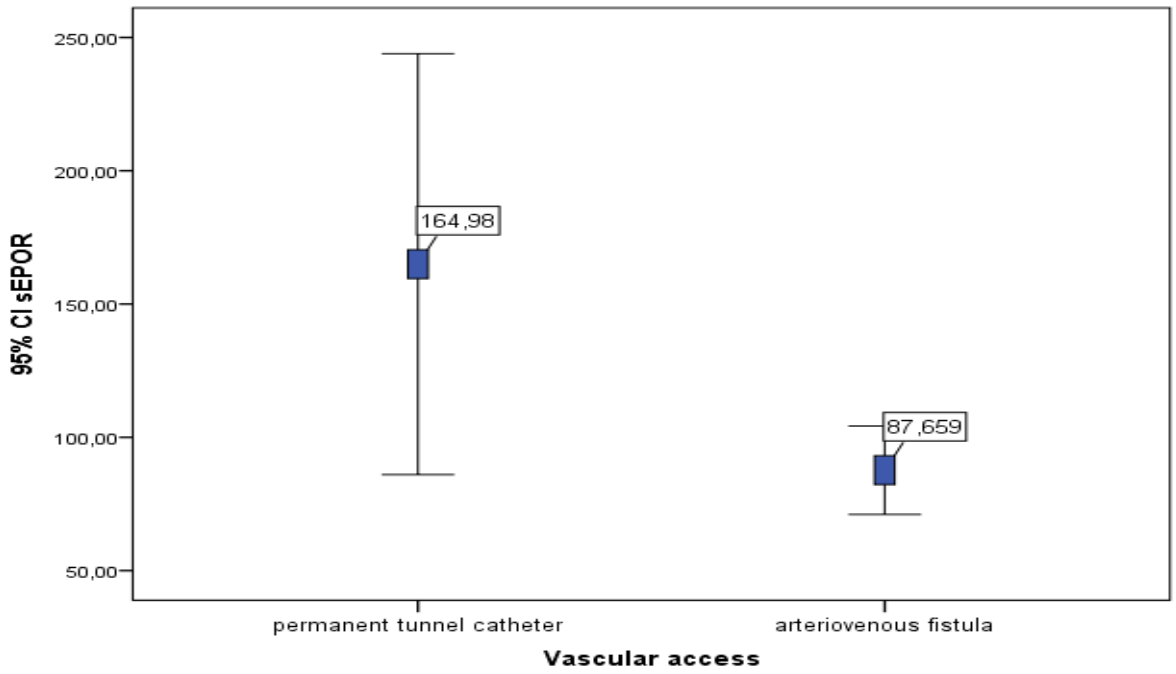

Fig. 49. Mean values of sEPOR according to vascular access

A study of the relationship between vascular access and sEPOR concentrations revealed a weak dependence (r = 0.245; p = 0.028) and a significant difference in sEPOR

values between patients with permanent tunnel catheter and arteriovenous fistula (p = 0.026). 49).

4.6. Study in the dynamics of the individual quality of life of patients with secondary hyperparathyroidism and anemia due to CKD

A quality of life was studied in 64 dialysis patients, with a mean age of 57.6 years, ranging in age from 33 years to 81 years (Fig. 50).

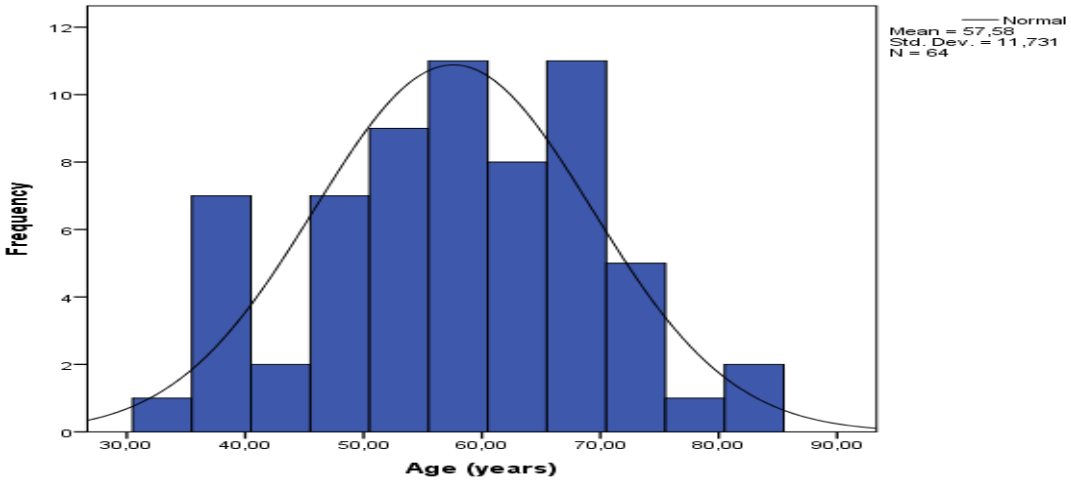

Fig. 50. Distribution by age of the patients

There is no difference in the distribution by gender, with a slight predominance of women (51.6%). Over ¾ (78.1%) of the respondents are unemployed.

The average duration of hemodialysis treatment is 61.02 months, with a minimum of 1 month and the maximum of 192 months. (Fig. 51).

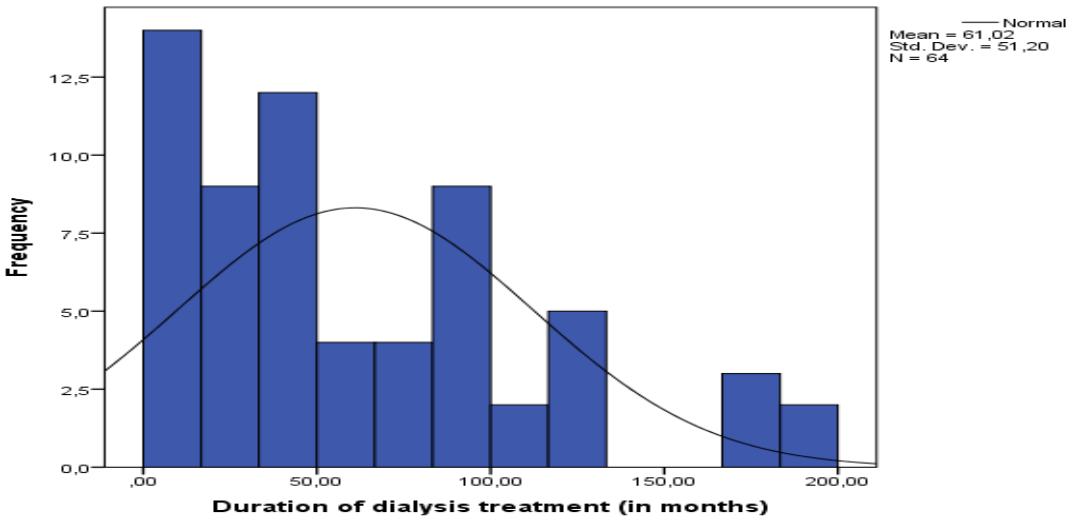

Fig. 51. Distribution according to the duration of hemodialysis treatment

More than half of the patients describe their quality of life as unsatisfactory (56.3%) and 31.3% as good. The remaining 12.4% cannot judge.

There was no significant difference in quality of life according to the age, duration of hemodialysis treatment and sex of the patients.

Patients with elevated ERI values were found to have an unsatisfactory quality of life (p = 0.05) (Fig. 52).

Another difference found was that patients with unsatisfactory QoL had high levels of iPTH compared to those who rated their quality of life as good (p = 0.037) (Fig. 53).

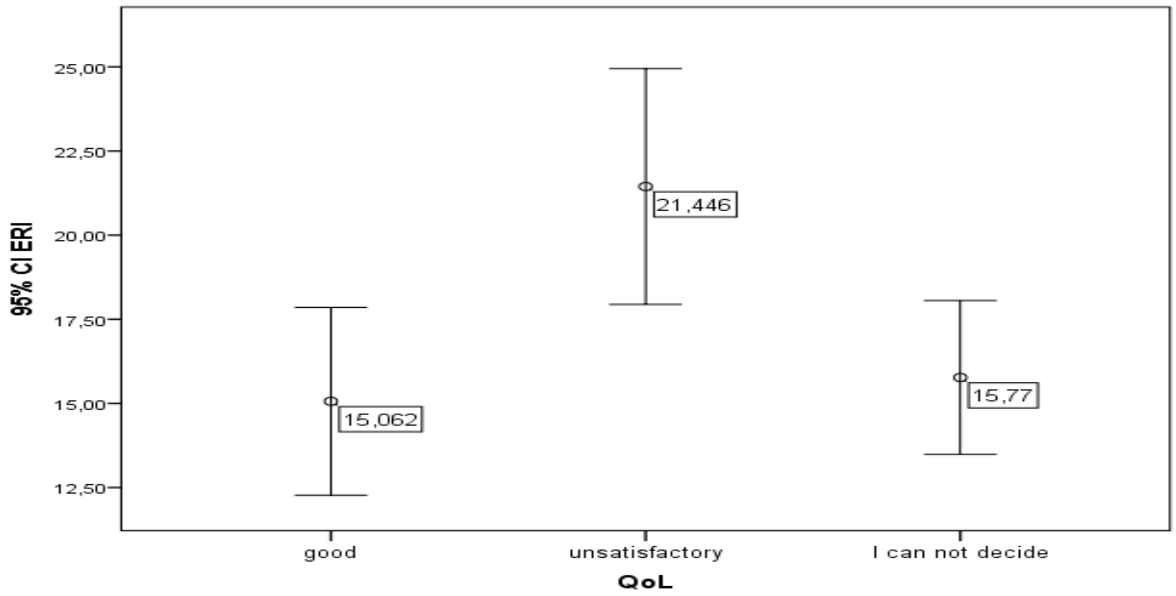

Fig. 52. ERI averages according to the QoL

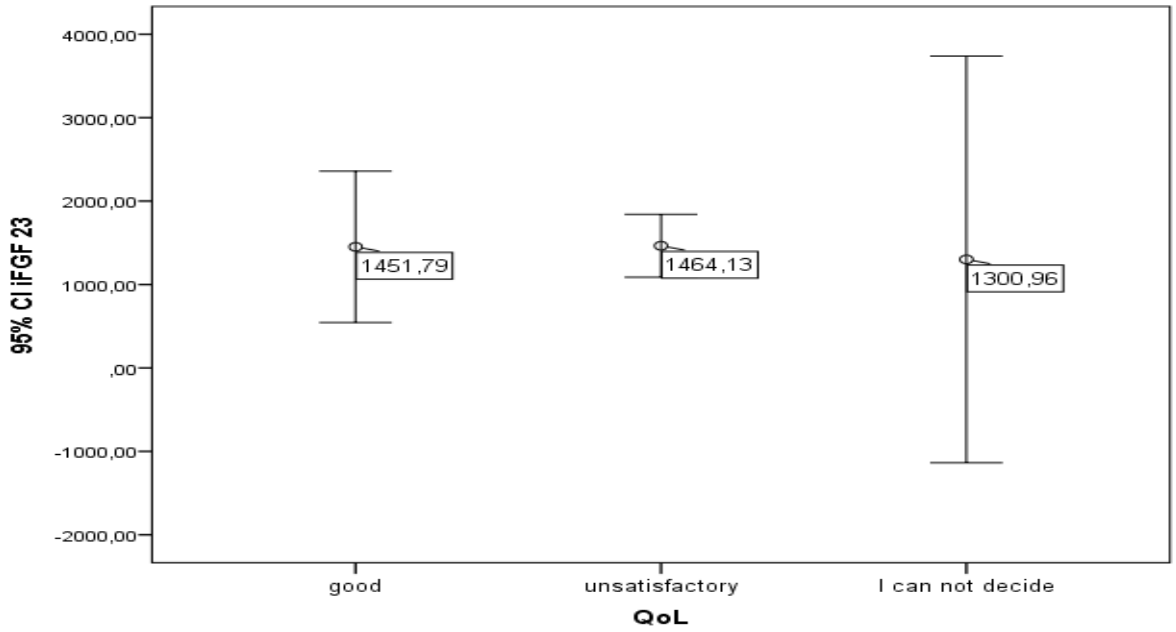

Fig. 53. Mean values of iPTH according to the QoL

Although no significant difference was found, patients with unsatisfactory QoL had higher sEPOR values (Fig. 54).

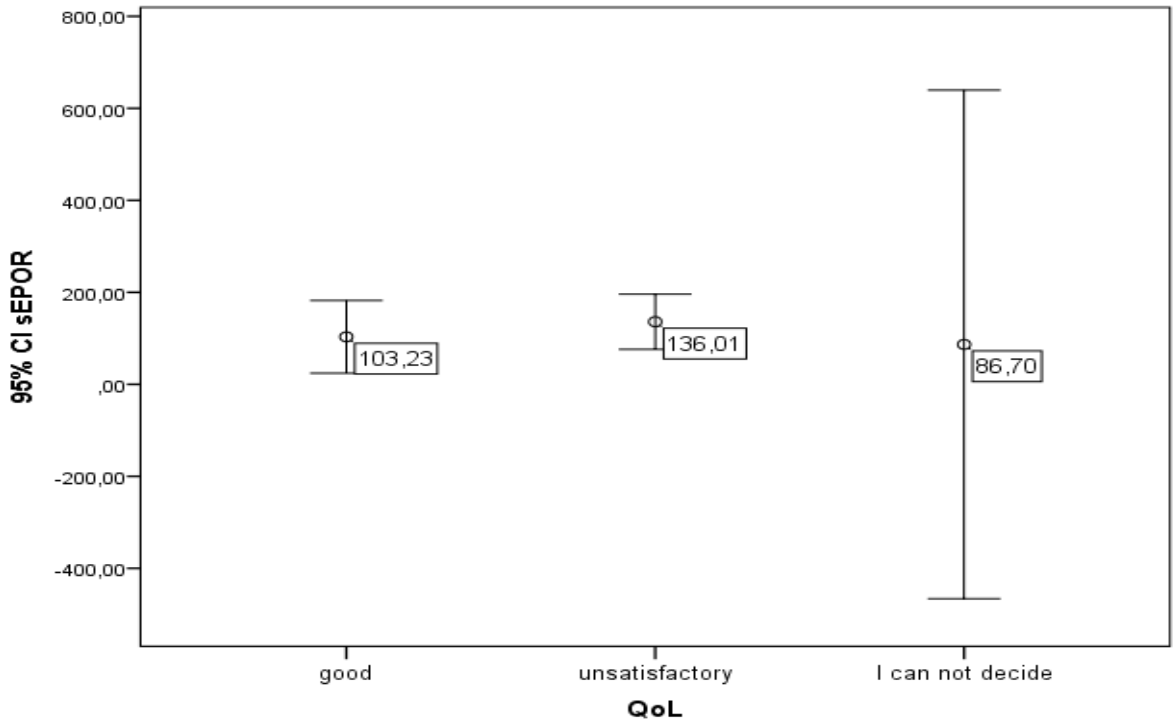

Fig. 54. Mean values of sEPOR according to QoL

Less than half of the patients (43.6%) reported having had any pain in the last four weeks, with a significant difference in QoL ($p < 0.05$) (Fig. 55).

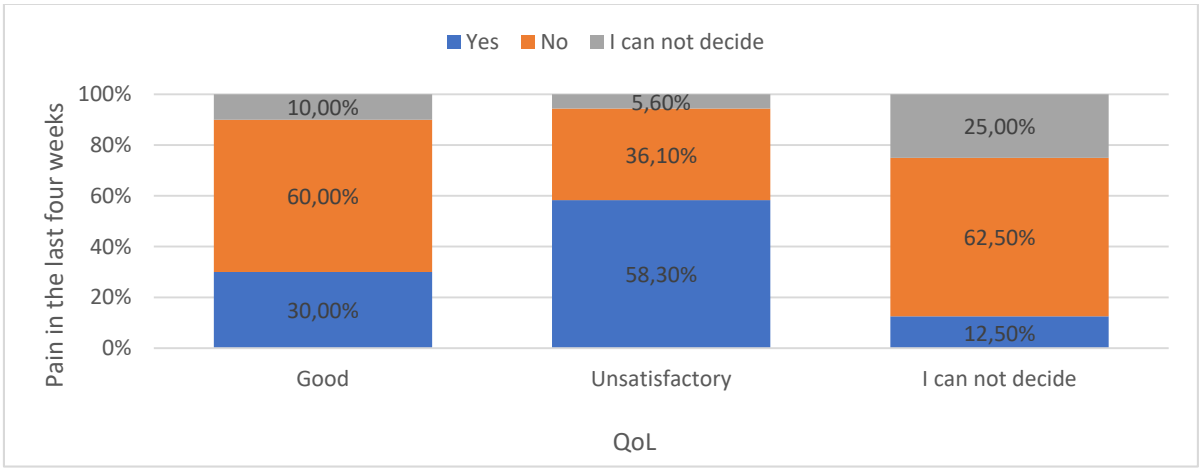

Fig. 55. Comparative analysis of the results of the presence of pain in the last four weeks and the assessment of QoL

In 32.8% of the respondents, the pain they experienced in the last four weeks had an impact on their daily activities, and a significant difference was found in their quality of life

(p = 0.05). 41.7% of the respondents, in whom the pain had an impact on their daily activities, defined their quality of life as unsatisfactory (Fig. 56).

Less than half (43.8%) of hemodialysis patients reported having light physical activity during the day, with 50.0% having moderate physical activity and only 6.2% having heavy physical activity. There is no difference in QoL according to the degree of the physical activity.

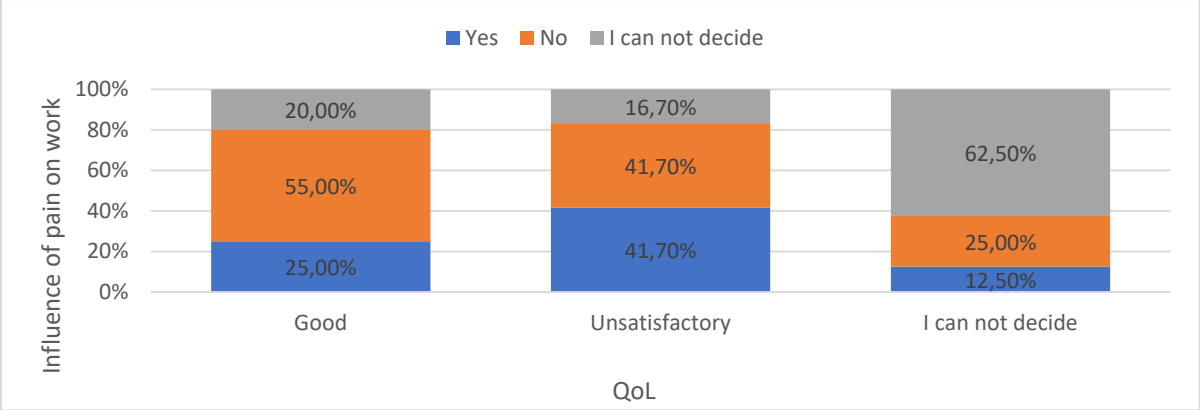

Fig. 56. Comparative analysis of the results of the influence of pain on the work and assessment of QoL

On the table 11 are presented the results of the influence of the studied indicators on pain.

Table 11. Influence of the studied indicators for erythropoietin resistance and SHPT on pain

Indicator	Pain		P value	R	P value
	Yes	No			
URR	67.94	66.87	0.877	0.014	0.941
ERI	23.35	16.64	0.039	-0.359	0.012
iPTH	1085.23	487.25	0.018	-0.444	0.018
iFGF 23	1541.23	1295.49	0.072	-0.101	0.638
sEPOR	100.99	129.15	0.276	0.136	0.274
antiEPOab	73.82	65.91	0.681	-0.164	0.377

The results in the table show that pain correlates with high ERI and iPTH values.

A significant countn of hemodialysis patients do not exercise (90.6%), 48.4% said that they have difficulties in performing normal daily activities, which changes their quality of life (p = 0.041) (Fig. 57).

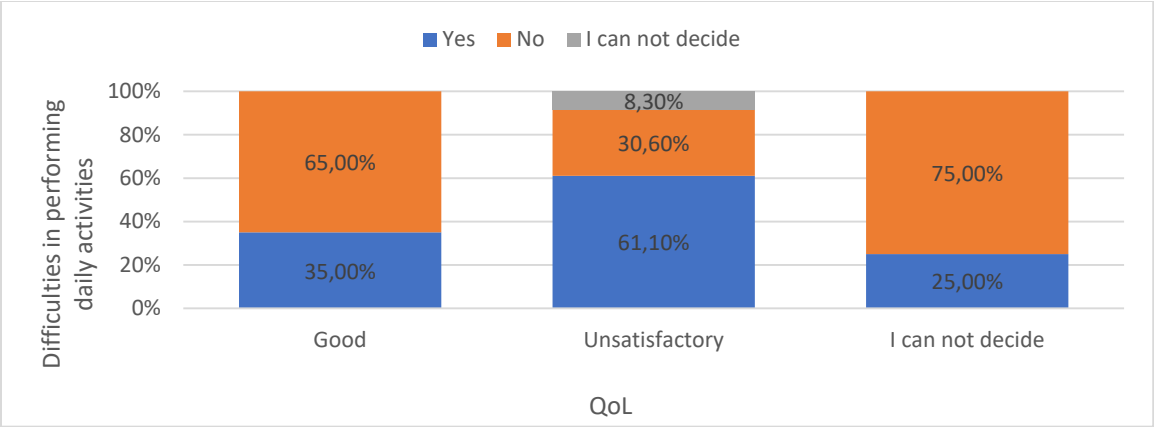

Fig. 57. Comparative analysis of the results of the difficulties in performing the daily activities and the assessment of the QoL

Although 81.3% said that they feel tired, this does not affect their quality of life. On the table 12 presents the influence of the considered indicators on fatigue.

Table 12. Influence of the studied indicators for erythropoietin resistance and SHPT on fatigue

Indicator	Fatigue		P value	R	P value
	Yes	No			
URR	68.14	59.43	0.045	-0.284	0.005
ERI	19.41	12.12	0.048	-0.241	0.032
iPTH	1423.55	408.00	0.033	-0.289	0.039
iFGF 23	1485.93	574.74	0.020	-0.271	0.020
sEPOR	126.92	49.50	0.047	-0.528	0.010
antiEPOab	84.77	59.33	0.062	-0.135	0.059

From the results presented in the table, it can be concluded that fatigue is associated with increased values of the studied indicators.

On the other hand, 48.4% said that they are restless, which influences their assessment of QoL, as a significant difference ($p = 0.009$) and dependence were found between the two factors ($r = 0.212$; $r < 0.05$) (Fig. 58).

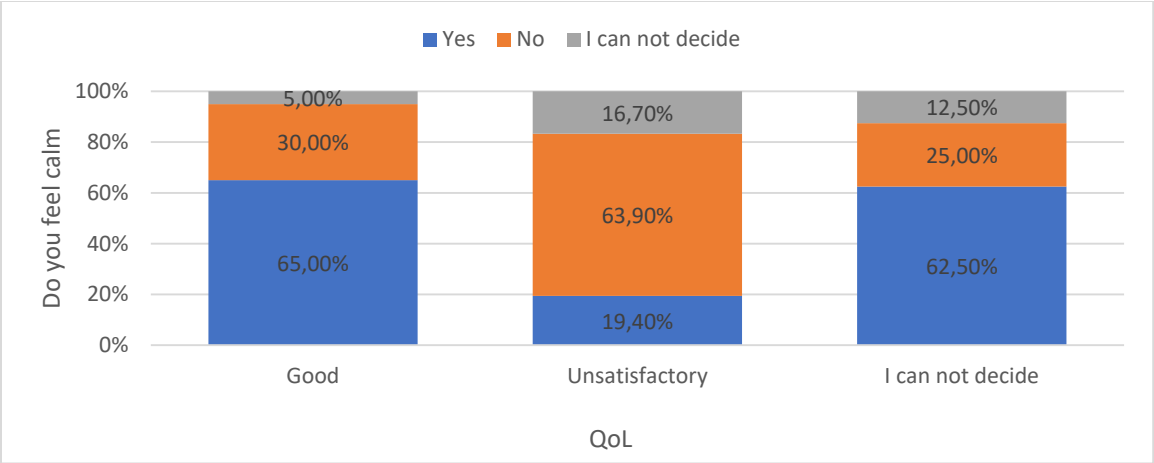

Fig. 58. Comparative analysis of the results of the feeling of calm and the assessment of QoL

Slightly more than half of the respondents (56.2%) reported that they felt more emotional compared to the period before the start of hemodialysis, with 57.8% admitting that they had a change in their intimate life. Less than ¾ (71.9%) state that they wake up often at night, but this does not affect their quality of life.

4.7. Development of an algorithm for the diagnosis of erythropoietin resistance in patients with CKD

In Fig. 59 is shown an algorithm for the diagnosis of erythropoietin resistance. Determination of reticulocyte count is a useful initial step in identifying patients with blood loss or hemolysis. In these patients, the reticulocyte count is often elevated. Such patients should be examined by fecal occult blood test, upper and lower gastrointestinal endoscopy or haemolytic screening (e.g. Coombs test), as appropriate. Blood loss should always be suspected in patients who require increasing doses of ESA to maintain a stable hemoglobin level, in patients whose hemoglobin levels fall despite treatment, and in those in whom iron replenishment cannot be achieved despite of the repeated intravenous applications.

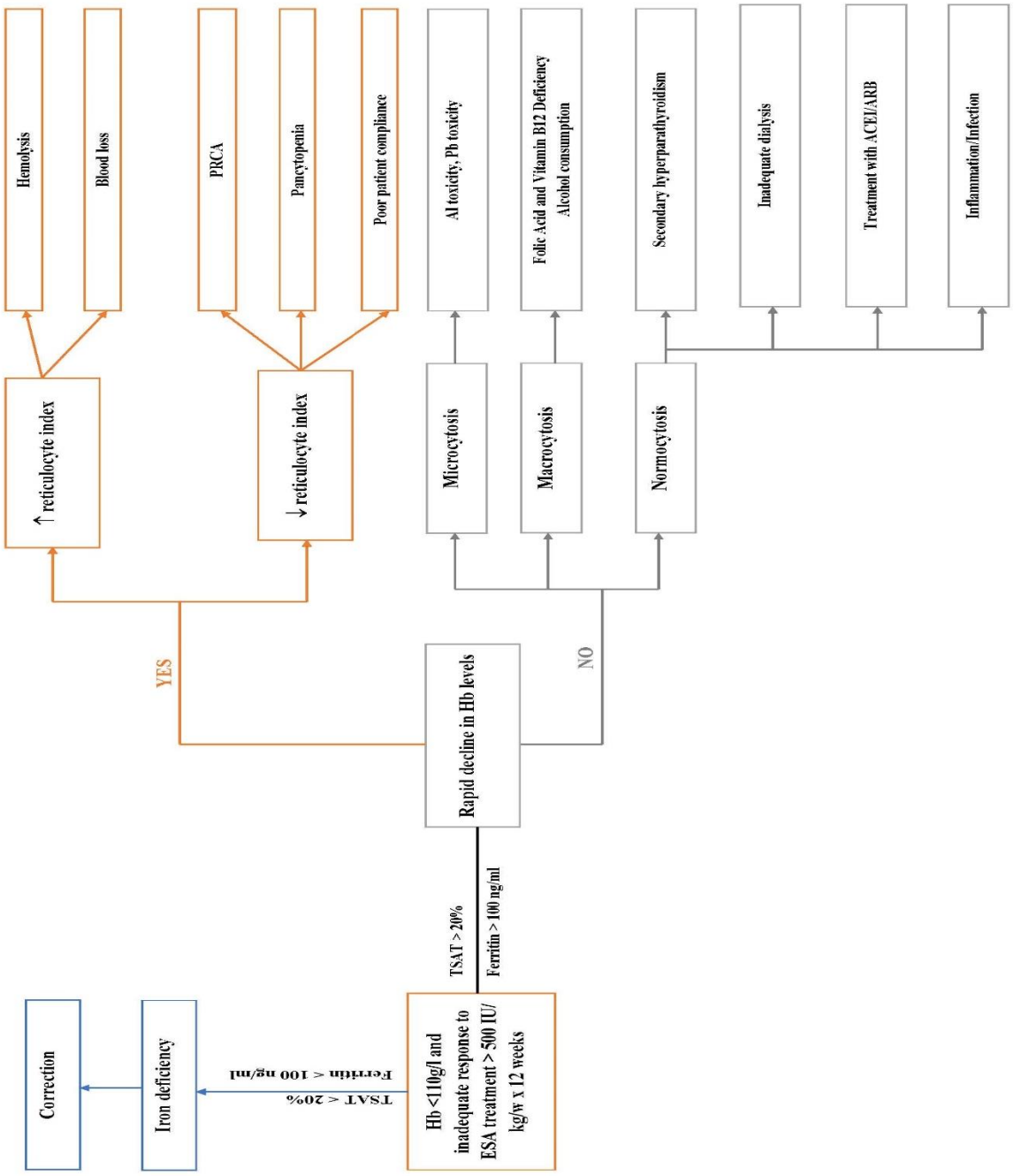

Fig. 59. An algorithm for diagnosis of erythropoietin resistance in patients with CKD

In patients with low reticulocyte counts (<40 × 10^9/L), it is first necessary to evaluate their compliance, especially when they are self-administering ESA. Excluding this potential, the two most common reasons for not responding to ESA are an iron deficiency and inflammation. We recommend testing for serum iron, TIBC, TSAT, ferritin and CRP. In the presence of an absolute or functional iron deficiency and serum ferritin levels <100 ng/mL, intravenous iron therapy is required. In the case of inflammation-related anemia, the cause must be identified (blood cultures, echocardiography, chest X-ray, abdominal computed tomography (CT), immunological status) and specific treatment as appropriate.

Neoplasms are another possible cause of an inadequate response to ESA treatment. Examination of tumor markers (eg PSA, Ca 19-9, Ca 125, Ca 15-3, AFP, CEA), upper and lower endoscopies of the gastrointestinal tract, CT of the chest and abdomen, consultation with a gynecologist and ultrasound/mammography of the mammary glands in women, consultation with a urologist in men with elevated PSA. If an iron deficiency and inflammation are ruled out, research and treatment for possible other causes should be undertaken: folic acid and vitamin B12 deficiency, secondary hyperparathyroidism (PTH), hypodialysis (Kt/V, URR), antibodies to erythropoietin, aluminum (a rare reason due to the use of modern, aluminum-free phosphate binders). Electrophoresis of hemoglobin is also considered (eg α-thalassemia or β-thalassemia). In the presence of antibodies to erythropoietin, a bone marrow test to detect PRCA is planned. If the patient is receiving ACEi and/or ARB therapy and there is no other clearly defined cause of erythropoietin resistance, it is recommended that the treatment with these drugs has to be discontinued (if the expected benefits outweigh the expected risks). Persistence of ESA hyporsponsiveness requires bone marrow examination to rule out other diseases (including myelodysplastic syndrome, myelofibrosis and pure red cell aplasia (PRCA)).

V. DISCUSSION

In their study, Schneider et al. found that an old age and male gender are predictors of ESA resistance. (321) These results are also confirmed by Panichi et al. - the patients belonging to the group with the highest ERI are older and predominantly male (279). Similarly, in an observational study of 1,710 patients, Lopez-Gomez et al. demonstrated that erythropoietin resistance was associated with an older age and the female is a risk factor for its development (218). In her prospective follow-up of 775 hemodialysis patients over a period of 12 years, Ashikova reported a statistically significant difference in erythropoietin resistance in women who did not receive ESA prior to hemodialysis compared to men ($p = 0.013$). The same follow-up also showed a statistically significant difference in the mean hemoglobin level in women who had not been treated with ESA prior to initiating hemodialysis treatment compared to men ($p = 0.047006$). (1) On the contrary to the data of these authors, the present study did not find a relationship between ERI and the age of the patients. On the other hand, 2/3 of men and women have elevated ERI values> 15.0.

An iron deficiency (absolute and functional) is a major risk factor for the development of erythropoietin resistance in patients with chronic kidney disease. A number of conditions contribute to iron deficiency in CKD, including blood loss, impaired iron absorption, uremic gastritis, and chronic inflammation. (178) (243) (17) Schneider et al. examined 1,015 hemodialysis patients and demonstrated that low serum iron and TSAT levels were associated with erythropoietin resistance, which is consistent with our findings. (321) In the European Survey on Anemia Management (ESAM) study, Jacobs et al. found inadequate iron stores in more than 50% of all patients treated with ESA (153).

Uremic syndrome, heart failure, persistent infections, biocompatibility of dialysis membranes, use of vascular catheters, accumulation of advanced glycation products, progressive reduction in glomerular filtration rate (eGFR), oxidative stress, hypoxia, immune dysfunction, intestinal dysbiosis, may contribute to the development of inflammation in CKD, with subsequent production of inflammatory cytokines. (370) (40) Cytokines have a direct effect on cell differentiation and mediate the induction of erythrocyte apoptosis. They also interfere with the erythropoietin-mediated signaling pathway by inhibiting the expression and regulation of specific transcription factors involved in the control of erythrocyte differentiation.(220) In hemodialysis patients, inflammation is associated with EPO resistance mainly because the inflammatory condition reduces the bone marrow response to ESA, altering the regulation of iron through increased regulation of hepcidin and /or causing hemolysis of erythrocytes. (327) A prospective cohort study conducted between 2009 and

2018, assessing 12,389 hemodialysis patients in 21 countries, reported that an increased CRP levels reduced the hemoglobin response to ESA treatment. Patients with elevated CRP levels have a faster decline in hemoglobin and need higher doses of ESA, leading to increased erythropoietin resistance. (170) In the present study, no dependence was found on iPTH levels, but a significant difference in CRP levels was found in patients in the pre-dialysis and dialysis groups ($p = 0.016$), with significantly higher values for this indicator for patients undergoing dialysis (26.67:17.93 mg/l). The mechanisms by which SHPT can cause anemia are not fully understood. High levels of PTH can affect red blood cell production either directly through the toxic effect of PTH on erythroid progenitors of bone marrow and increased hemolysis or indirectly by stimulating bone marrow fibrosis (143). This theory is supported by the improvement of anemia and the reduction of bone marrow fibrosis after parathyroidectomy (228). PTH is considered a uremic toxin that potentially inhibits EPO synthesis, reduces erythrocyte survival and causes myelofibrosis, thus suppressing hematopoiesis. In addition, new data suggest that FGF-23 is also involved in inefficient erythropoiesis in CKD (65), (335), (237).

Coe et al. have shown that FGF-23 negatively regulates erythropoiesis by inhibiting EPO production and erythropoietin receptor expression (65). In addition, an experimental study by Singh showed that FGF-23 directly stimulates an inflammatory response. Because chronic inflammation is a common cause of anemia, FGF-23-induced inflammation may also contribute to renal anemia and an inadequate response to EPO. (335) In support of these findings, the Chronic Renal Insufficiency Cohort Study (CRIC) Mehta et al. demonstrate significant associations of elevated FGF-23 levels with changes in hemoglobin levels over time and the development of anemia. In the present study, no relationship was found between iFGF-23 and CRP levels, but a significant difference was found in the studied groups ($p < 0.05$). In patients in the pre-dialysis group there was a decrease in CRP levels in iFGF-23 <1880.14pg/ml, while in patients in the dialysis group we observed an increase in CRP levels in iFGF-23 <1109.19pg/ml, after which CRP slowly began to decrease, but remain above the reference limit. (237)

The association between hyperparathyroidism and the ESA response has been evaluated in a number of studies. Amnuay et al. analyzed 43 patients with CKD, finding that those with high levels of iPTH had significantly lower hemoglobin levels and received higher doses of ESA compared to the group with normal levels of iPTH (Hb 8.29 vs. 9.24 mg/dL, $p = 0.032$ and ESA dose 16,352.94 UI/week vs. 12,444.44 UI /week, $p = 0.024$). Multivariate step analysis found that serum phosphate concentrations were significantly associated with

lower Hb levels (p = 0.01 and p = 0.013, respectively). In addition, hemoglobin levels correlated inversely with iPTH and serum phosphate (r = -0.54, p <0.001 and r = -0.47, p = 0.005; respectively). Mineral imbalance is an important factor associated with erythropoietin resistance in CKD. Hyperphosphatemia and SHPT correlate significantly with low hemoglobin levels. (15) In the present study, a significant difference was found in the values of PTH according to ERI, which are significantly higher in patients in the dialysis group. There has been a direct proportional moderate relationship between ERI and FGF-23 (r = 0.3, p = 0.02). There was also a significant difference in phosphates according to ERI between patients in the two groups (p = 0.002) (15). Boronat et al. studied 382 patients with anemia and advanced CKD in the predialysis stage, using logistic regression models to compare hemoglobin levels with those of serum calcium, phosphate, 25(OH)D3 and alkaline phosphatase. In an uncorrected logistic regression model, serum calcium and 25(OH)D3 (negative) and phosphorus (positive) concentrations were significantly associated with anemia. In a fully corrected multivariable model, the OR for anemia was 0.29 (95% CI 0.16-0.49; p <0.0001) for every 1 mg/dl increase in serum calcium and 2.19 (95% CI 1, 55-3.15, p <0) for every 1 mg/dl increase in serum phosphate. Female sex and lower serum albumin levels are also independently associated with anemia. In conclusion, circulating calcium and phosphate levels were found to be strongly associated with anemia in patients with advanced CKD (pre-dialysis stages). (43) There was a significant difference in ERI and phosphate levels between dialysis and pre-dialysis patients, with those on dialysis having higher phosphate levels.

The association between erythropoietin resistance and iPTH values was also studied by Al-Hilali et al., who studied 118 patients with end-stage CKD (83 on hemodialysis and 35 on continuous ambulatory peritoneal dialysis (CAPD)). They found a significantly higher ERI in hemodialysis patients than in peritoneal dialysis at iPTH <16 pmol/l (p = 0.002) and iPTH between 16-32 pmol/l (p = 0.012). There was no significant difference between hemodialysis and peritoneal dialysis patients with iPTH higher than 32 pmol/l. A statistically significant difference (p = 0.04) was found between low Hb (<11g/l) and iPTH > 32 pmol/l. A statistically significant difference (p = 0.044) was also found between hematocrit (Hct) <33% and iPTH > 32 pmol/l. (10)

They confirmed that SHPT was a predictor of erythropoietin resistance in dialysis patients, and that peritoneal dialysis patients had lower levels of iPTH and ERI and needed lower doses of recombinant erythropoietin for their treatment. In another study, Kimata et al. concluded that higher serum calcium and phosphate levels are independently associated with

better control of anemia (182). Inhibition of erythropoietin progenitors and changes in tissue sensitivity to erythropoietin and PTH are thought to have a direct negative effect on the reduced response to erythropoietin treatment (241). In addition, plasma erythropoietin levels increase dramatically 1-2 weeks after surgical parathyroidectomy (PTx) (363)

In patients undergoing parathyroidectomy, Mandolfo et al. observed a 20% increase in hemoglobin and a 34% decrease in EPO dose in patients after PTx. (228) In a similar study, Lee et al. obtain comparable results. In 32 hemodialysis patients, three months after parathyroidectomy, there was a decrease in the dose of ESA required to maintain target hematocrit levels by 29% from baseline ($p < 0.05$). (205) Four separate multivariate analyzes conducted in the USA, Canada and Sweden evaluated hemodialysis patients who did not undergo parathyroidectomy and found associations between higher iPTH, lower hemoglobin concentrations (<11 g/dL) and the need of higher weekly doses of ESA (IU/kg /week). (5)

In a randomized controlled trial in South Africa, Benjamin et al. studied forty patients with end-stage CKD treated with recombinant EPO Beta (Recormon) at weekly doses between 2,000 IU and 18,000 IU depending on the severity of the anemia. Antibodies to EPO were detected in almost all patients, with the highest antibody titers being in patients receiving the highest weekly dose of EPO (18,000 IU) - OR = 3,975 (E +6) (8,233–1,920 E +12) ($P = 0.026$). Low hemoglobin levels are also associated with higher weekly ESA requirements and also revealed that serum EPO levels, gender or age were not associated with any significant variation in serum antibody concentrations. (36)

In its study, which included 128 dialysis patients, Rahbar et al. did not establish a correlation between age, sex, cause of CKD, duration of hemodialysis, hemoglobin level, dose and duration of rHu-EPO treatment, and anti-EPO antibody levels. (297) In the present study, elevated levels of erythropoietin antibodies were found in both groups of patients, with this indicator having questionable results, which we believe is due to the methodology used in the study.

Khankin et al. have measured sEPOR by ELISA among 697 dialysis patients, most of whom had low sEPOR levels (less than or equal to 100 pg/ml). The mean levels of sEPOR at the beginning of dialysis treatment were 2437 ± 1299 pg/ml in the high group ($n = 36$) and 112 ± 111 pg/ml in the low group ($n = 661$) ($p < 0.001$). The mean values were 2147 pg/ml (interquartile range 1400–3445 pg/ml) and 69 pg/ml (interquartile range 62.5–101 pg / ml), respectively.

They compared sEPOR concentrations with ESA doses, finding that ESA doses not only differed over time ($P < 0.001$), but also according to sEPOR concentrations on the 90th

and especially on the 180th day after initiation of hemodialysis treatment (P = 0.038). In the low sEPOR group, there were slightly higher hemoglobin levels, although patients received lower weekly doses of ESA on day 180. The authors concluded that patients who received higher cumulative doses of erythropoietin during the study period had progressively higher baseline levels of sEPOR. The need for ESA in high versus low sEPOR groups is approximately 3-fold higher (OR 2.8, 95% CI 1.3–6.4). (175) In the present study, the analysis of the relationship between Hb and sEPOR found an inversely weak relationship (r = -0.201; p = 0.036), which shows that patients with lower sEPOR values have higher Hb levels. A study of the relationship between vascular access and sEPOR concentrations revealed a weak dependence (r = 0.245; p = 0.028) and a significant difference in sEPOR values between patients with permanent tunnel catheter and arteriovenous fistula (p = 0.026). Differences in sEPOR concentrations were also found according to ESA doses, where low doses were associated with high doses of sEPOR in pre-dialysis patients, while higher doses of sEPOR were associated with the need for higher doses of ESA.

Elevated sEPOR levels have been associated with reduced quality of life and the presence of pain and fatigue in dialysis patients. Another difference found in the values of this indicator is related to the levels of iFGF-23, where low values of iFGF-23 correlate with high levels of sEPOR in patients in the dialysis group, and increased values of iFGF-23 correlate with increased sEPOR values in patients in the pre-dialysis group.

The ESA response has been shown to be positively related to both the duration of dialysis (318) and the dialysis dose administered as determined by URR (145) or Kt/V. (216), (250) In its prospective study in 20 patients on hemodialysis Ifudu et al. found that an increase in URR from 60.7% to 72% over 6 weeks was accompanied by an increase in hematocrit from 28.4 ± 0.78% to 32.3 ± 0.71% (p = 0.002). On the contrary, in 20 control patients with equivalent baseline URR levels who did not change dialysis modality, the mean hematocrit did not change (28.2 ± 0.84% to 26.3 ± 0, 85%; P = 0.175). (145) However, Locatelli et al. did not demonstrate the benefit of high-flux hemodialysis on ESA sensitivity in a small (n = 84), short-term (12 weeks) randomized controlled trial. (215) In our study, we found that the duration of hemodialysis treatment was moderately proportional to ERI (r = 0.373; p = 0.008). The longer the hemodialysis treatment, the greater the risk of developing erythropoietin resistance. ERI correlates moderately inversely with URR (r = -0.305; p = 0.031), which means that inadequate dialysis treatment is also associated with an increased risk of developing erythropoietin resistance.

A review of the literature shows that the main socio-demographic factors related to the quality of life of dialysis patients are old age, female gender, obesity, lack of education, unemployment, duration of hemodialysis treatment and concomitant diseases (257), (313). The current study did not find a relationship between quality of life and gender, age, duration of hemodialysis and employment, with a predominance of women (51.6%) and unemployed (78.1%).

In his multicenter cross-sectional study, Sa'ed H. Zyoud et al. studied the quality of life in 267 dialysis patients and found that there was a significant negative association between HRQOL with age, the total number of chronic comorbidities and the number of medications taken. A significant positive relationship is found between HRQOL and males, educational attainment and living in rural areas. (313) In the present study, unsatisfactory QoL in dialysis patients correlated with high levels of ERI and PTH. It was found that pain also correlates with high levels of these two indicators, and fatigue is associated with elevated values of ERI, iPTH, iFGF - 23, sEPOR and antibodies to erythropoietin.

Dąbrowska-Bender et al. examined the quality of life of 140 patients with end-stage CKD, finding that hemodialysis patients had the following symptoms more often than peritoneal dialysis: sleep disturbance (very common: 30%, common: 19%), feeling pain (very common: 15%, common: 17%) and difficulties in sexual life (very common: 4%, common: 6%). At the same time, there are significant differences between hemodialysis patients and peritoneal dialysis patients in terms of sleep disturbance and pain. (69) The present results also support the hypothesis that pain lowers Qol in hemodialysis patients, with 58.3% of those with unsatisfactory Qol having pain in the last four weeks.

Sleep disorders have an adverse effect on patients' ability to perform their daily activities, to concentrate, increase the incidence of depression and lead to tension in family relationships.

Petkova et al. investigate sleep disorders in 27 patients on hemodialysis at the Clinic of Nephrology and Dialysis, University Hospital "St. Marina " Varna. It was found that sleep disorders are represented by a high relative share in these patients. In addition, a statistically significant difference was found between the hemodialysis group and patients with breath-disorderes during sleeping (97.3%) in terms of snoring ($p < 0.001$) (2). The current study found that 71.9% of people have trouble sleeping, but this does not affect their quality of life.

Todorova et al. conduct a program aimed at raising patients' awareness of the necessary diet, care for vascular access and the need to maintain social activity. They compared the assessment of quality of life in 65 patients on hemodialysis, finding significant

differences before and after the program ($p < 0.004$) in the ability to perform normal activities. A comparative analysis of the results revealed a significant difference regarding to energetic feeling ($p < 0.002$) and quality of life ($p < 0.001$) (3). In the present study, there is also a difference in QoL in patients who are unable to perform their daily activities due to the presence of pain.

Ishiwatari et al. studied the quality of life among 892 hemodialysis patients over the age of 60 in Japan, noting that physical quality of life deteriorated with increasing duration of dialysis treatment. Elderly hemodialysis patients are thought to be at higher risk of worsening HRQOL and to be in greater need of social and psychological support from medical staff and their families (152).

The quality of life of patients suffering from CKD is also formed on a base of social and family relationships. Lack of support and acceptance from family and friends has a negative impact on patients' health through lower self-esteem and feelings of hopelessness, which worsens mood, leads to depression, feelings of resignation and inferiority. In the current study, 56.2% of respondents said that they had an increase in emotionality compared to the period before starting dialysis treatment, and another 57.8% had a change in intimate life, which did not lead to a change in their quality of life.

In their study, Malindretos et al. studied 156 hemodialysis patients (50 patients with PTH> 300 pg / ml and 106 patients with PTH <300 pg / ml), with no significant differences in the presence of comorbidities between groups. They found that patients with elevated PTH had a worse overall score for the pain component ($p = 0.036$) and a worse overall score for the physical component ($p = 0.029$). (226)

Liang et al. investigated the clinical effect of high doses of calcitriol in patients with secondary hyperparathyroidism and end-stage chronic renal disease. Patients were divided into two groups - a control group treated with conventional doses of calcitriol and a group treated with a high dose of calcitriol. There was no significant difference in quality of life between the two groups before treatment, and then the quality of life in the two groups improved, with the group treated with high doses of calcitriol being significantly higher ($P < 0.05$). (208)

CONCLUSIONS

1) There is a significant relationship between iPTH and ERI in patients in the dialysis group, which is rather negative.
2) ERI correlates with iFGF-23 and hemoglobin levels, and the risk of developing EPO resistance is significantly higher in dialysis patients.
3) Biomarkers for mineral-bone metabolism (iPTH and iFGF-23) correlate negatively with BMI and positively with folic acid.
4) There is a significant difference in the treatment regimens of patients in the pre-dialysis and dialysis stages according to SPHT and anemia.
5) Iron therapy correlates strongly with dialysis treatment, with a strong relationship between ERI and ESA dose.
6) Individual quality of life correlates negatively with the duration of dialysis, pain and a sense of calm.

CONTRIBUTIONS

Theoretical contributions

1) Non-invasive biomarkers in patients in the risk groups of CKD (pre-dialysis and dialysis stages) have been studied and followed, which can be implemented in practice regarding the diagnostic and treatment process.
2) A detailed review of the literature on the nature of erythropoietin resistance and the factors that determine it has been done.
3) For the first time such a study was conducted in the country to determine the level of erythropoietin resistance by direct examination of the titer of antibodies to erythropoietin, as well as to determine their dependence on other biomarkers.
4) The association between secondary hyperparathyroidism and erythropoietin resistance in patients with CKD has been demonstrated.
5) The individual quality of life of patients with erythropoietin resistance and secondary hyperparathyroidism was assessed.

Practical contributions

1) Biochemical indicators have been studied, which are not routinely analyzed among the patients monitored in the Clinic of Nephrology of the University Hospital "St. Marina"- iFGF-23, level of folic acid and vitamin B12, as well as those not studied so far in the country (antibodies to erythropoietin, soluble erythropoietin receptor).
2) An algorithm for the diagnosis of erythropoietin resistance in patients with CKD has been developed and proposed.
3) The adequacy of the therapy in relation to the anemia, secondary hyperparathyroidism and the individual quality of life among patients in pre-dialysis and dialysis stages of CKD was assessed.

PUBLICATIONS RELATED TO THE DISSERTATION

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