Medical University - Varna
„ Prof. Dr. Paraskev Stoyanov”

Faculty „Medicine“
Department of Second Department of Internal Medicine

DR. PETAR PLAMENOV PETROV

MONITORING AND ASSESSMENT OF NUTRITIONAL STATUS AND MARKERS OF THE INFLAMMATORY PROCESS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

SUMMARY

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

Supervisor:

Prof. Dr. Svetla Vasileva Staykova, MD, PhD, DSc

Scientific consultant:

Assoc. Prof. Dr. Lili Slavcheva Grudeva, MD, PhD

Varna, 2022

The dissertation contains 150 standard pages and is illustrated with 6 tables, 42 figures and 3 appendices. The literature reference includes 183 literary sources, of which 2 in Cyrillic and 181 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.

External members:

1. Prof. Dr. Boryana Petrova Deliyska, MD, PhD, DSc
2. Prof. Dr. Iliya Dimitrov Popov, MD, PhD
3. Assoc. Prof. Dr. Velislava Dimitrova Dimitrova, MD, PhD

Spare external member:

1. Assoc. Prof. Dr. Vasil Ivanov Vasilev, MD, Ph.D.

Internal members:

1. Assoc. Prof. Dr. Kiril Svetoslavov Nenov, MD, PhD
2. Assoc. Prof. Dr. Irina Ivanova Ivanova, MD, PhD

Spare internal member:

1. Assoc. Prof. Dr. Trifon Chervenkov, MD, Ph.D

The official defense of the dissertation will take place on February 24, 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

ACR	Albumin to creatinine ratio
ADMA	Asymmetric dimethylarginine
APOL1	Apolipoprotein L1
ARIC	Atherosclerosis Risk in Communities Study Description
BMI	Body mass index
BNP	B-type natriuretic peptide
BTP	B-trace protein
CD14	Cluster of differentiation 14
CRP	C-reactive protein
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
FGF 23	Fibroblast growth factor - 23
GBD	Global burden of Disease
GF	Glomerular filtration
HDL	High - density lipoprotein
HIV	Human immunodeficiency virus
IgA	Immunoglobulin A
IGF-1	Insulin-like Growth Factor - 1
ISRNM	International society of renal nutrition and metabolism
KDIGO	Kidney Disease Improving Global Outcomes
KIM – 1	Kidney injury molecule - 1
L- FABP	Urinary L-type fatty acid binding protein
LDL	Low – density lipoprotein
MDRD	Modification of diet in renal disease study
MIA syndrome	Malnutrition – inflammation - atherosclerosis
NAD +	Nicotinamide adenine dinucleotide +
NAG	N – Acetyl – b - D – glucosaminidase
NGAL	Neutrophil gelatinase-associated lipocalin
NIH	National Institute of Health
NMN	Nicotinamide mononucleotide
NAMPT	Nicotinamide phosphoribosyltransferase protein

PBEF-1	Pre-B-cell colony-enhancing factor - 1
PEW	Protein-energy wasting
PRPP	Phosphoribosyl pyrophosphate
PTH	Parathyroid hormone
QOL	Quality of life
SDMA	Symmetric dimethylarginine
sEPOR	Erythropoietin Receptor
sVCAM-1	Soluble vascular cell adhesion protein - 1
T3 и T4	Thyroid hormones
TGF-b	Transforming growth factor - beta
TGF-b1	Transforming growth factor beta -1
TNF	Tumor necrosis factor
TSH	Thyroid stimulating hormone
VCAM – 1	Vascular cell adhesion protein - 1

I. INTRODUCTION

Chronic kidney disease (CKD) is a serious public health problem that can lead to end-stage renal disease, increased cardiovascular morbidity and mortality. Identifying the predisposing factors for CKD is essential, as some of them can be modified, prevented or slowed down.

Recent studies suggest that the average incidence of CKD worldwide is about 10-12% with a tendency to progressively increase. In the 21st century, there is talk of an "epidemic" of chronic kidney disease. It has been found that each year about 300-400 patients per 1,000,000 population reach the terminal stage of CKD and need to start dialysis treatment.

Visfatin, also known as pre-B-cell colony enhancing factor-1 (PBEF-1) or nicotinamide phosphoribosyltransferase (NAMPT) is adipokine.

Visfatin plays an important role in innate immunity. It is secreted by activated lymphocytes, monocytes and neutrophils and stimulates the secretion of IL-6 by P38 mitogen-activated protein kinase (MAPK) and MAPK kinase-1 pathways. It also induces the expression of inflammatory mediators in humans on endothelial cells through the nuclear factor (NF) -κB pathway.

Studies published to date have shown that elevated serum visfatin levels can be considered as a marker of endothelial dysfunction and thus participate in predicting the incidence of cardiovascular disease in patients with chronic kidney disease.

II. AIM, TASKS

2.1. Aim

The aim of the present dissertation is to establish a correlation between the new non-invasive biomarker (Visfatin) and the nutritional status / inflammatory process, in view of its prognostic value in patients with chronic kidney disease.

2.2. Tasks

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

1. To characterize the modern non-invasive biomarker (Visfatin) related to the inflammatory process and its diagnostic value.
2. To evaluate the practical significance of the modern non-invasive biomarker (Visfatin), sensitive to the inflammatory process.
3. To look for a correlation between Visfatin with the nutritional status of patients with chronic kidney disease.
4. To compare the levels of the new non-invasive biomarker with indicators characterizing the inflammatory process in patients with chronic kidney disease.
5. To monitor changes in individual quality of life depending on nutritional status and the accompanying inflammatory process.

III. MATERIAL AND METHODS

3.1. Object and scope of the study

The subject of the study are a total of 80 patients with chronic kidney disease, divided into two groups - predialysis (30 patients) and hemodialysis treatment (50 patients) 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 without concomitant malignancies
- Persons who have signed an informed consent

б) Criteria for exclusion of persons

- Persons under 18 years of age
- Persons with concomitant malignancies
- Persons with active bleeding from the digestive system
- Persons who have not signed an informed consent

3.2. Research period

The current comprehensive diagnostic and therapeutic study was conducted for a period of 6 months - April - October 2021.

3.3. Research methodology

3.3.1. Laboratory researches

The following laboratory parameters were studied:

- Intact parathyroid hormone (iPTH)
- Intact fibroblast growth factor -23 (iFGF-23)
- Folic acid level
- Vitamin B12 level
- Serum iron level
- sEPOR level
- CRP

- Albumin

Indicator	Apparatus	Method
Serum Iron	Siemens ADVIA 1800	Colorimetric method with Ferrozine.
Albumin	Siemens ADVIA 1800	Colorimetric method with Bromocresol green BCG
PTH	Immulite 2000 XP	Chemiluminescent immunoassay

Serum levels of: vitamin B12, folic acid, Human EPOR levels and intact fibroblast growth factor-23 (iFGF-23) were examined in all participants using the sandwich ELISA method.

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 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.

3.3.1.4. 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.

3.3.1.5. Determination of serum levels of Visfatin

Visfatin levels were determined in blood serum using the ready-made Human VF (Visfatin) ELISA kit, catalog number HUES02741, from Assay Genie, Ireland, with a sensitivity (detection threshold) of 0.188 ng / ml and a linear range of 0.313 - 20 ng / Jr. 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 Visfatin was performed according to the manufacturer's protocol, diluting the samples 5 times. Visfatin concentration in ng / ml was calculated based on the relevant standards using 5 parametric logistic nonlinear regression using MikroWin software version 4.31.

The results are reported by measuring the optical density, dynamic range and sensitivity of the obtained solutions, on the basis of which, according to mathematical formulas, standard curves are prepared, showing the corresponding serum concentrations.

3.3.2. 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)
- ROC curve analysis to determine the threshold value of Visfatin.

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 modern non-invasive biomarker (Visfatin) related to the inflammatory process and its diagnostic value

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 patients

Indicator		Predialysis group(n=30)	Dialysis group (n=50)	P value
Age (years)	mean±SD (range)	64.33±13.66 (26-85)	62.32±13.51 (36-88)	>0.05
Gender	male	50.00%	58.00%	>0.05
	female	50.00%	42.00%	
Diagnosis	Diabetic nephropathy	16.67%	20.00%	>0.05
	Hypertensive nephropathy	53.33%	46.00%	
	Chronic glomerulonephritis	20.00%	24.00%	
	Chronic tubulointerstitial nephritis	-	6.00%	
	Autosomal dominant renal polycystosis	-	4.00%	
	Chronic pyelonephritis	10.00%	-	
Urea	mean±SD (range)	19.12±6.53 (8.00-40.00)	25.44±9.94 (10.20-57.10)	0.003
Creatinine	mean±SD (range)	296.70±96.13 (148.0-565.0)	788.60±197.80 (460.0-1399.0)	<0.001
Serum iron	mean±SD (range)	10.52±4.66 (1.10-23.60)	9.88±5.46 (1.20-30.10)	>0.05

There is no significant difference in the distribution of patients by age and sex and allows for comparability of results in subsequent analyzes.

High urea levels correlate moderately with the progression of CKD ($r = 0.331$; $p = 0.003$).

It was found that the progression of CKD strongly correlated with high creatinine levels ($r = 0.822$; $p < 0.001$), and in 67.5% of cases high creatinine levels were associated with CKD progression and renal replacement therapy.

When comparing the mean values of Visfatin between the two groups, no significant difference was found in the values between the two groups - dialysis group (21.7 ng / ml), compared to pre-dialysis group (26.7 ng / ml) (Fig. 1 and Fig. 2).

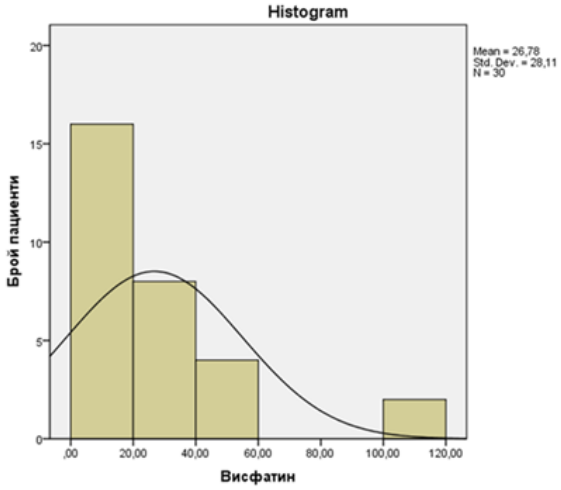

FIG. 1. Mean values of Visfatin and distribution of patients in the predialysis group

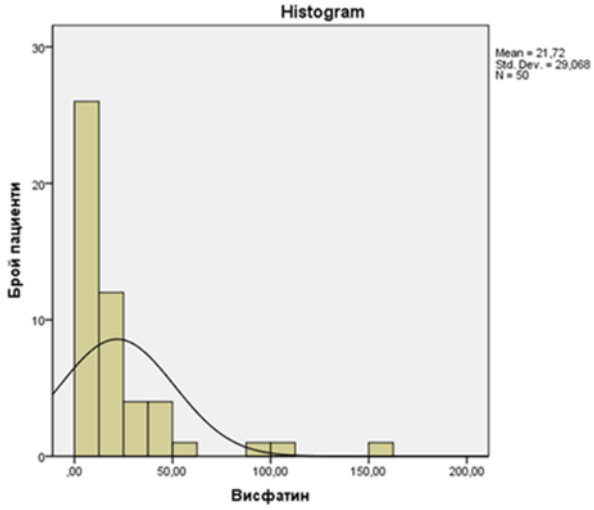

FIG. 2. Mean values of Visfatin and distribution of patients in the dialysis group

Due to the lack of uniform reference limits for Visfatin, a threshold of 16.92 ng / ml (AUC = 0.612 (0.485-0.739); p <0.05) was found, with a distinction between patients in the two groups with a sensitivity of 55.2% and a specificity of 57.1%. Fig. 3).

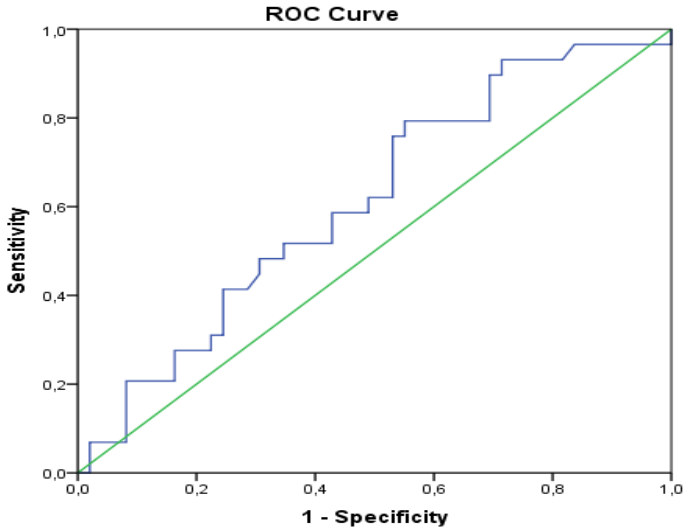

FIG. 3. ROC curve analysis to determine the threshold value of Visfatin

When determining the Visfatin threshold, values above 16.92 ng / ml were found to correlate moderately with earlier stages of CKD, while lower values were associated with dialysis (r = 0.299; p <0.05).

In the study of Visfatin values it was found that there is a significant difference between men and women in the predialysis and dialysis groups (p <0.01), and in women in both study groups significantly higher values of the marker were observed (Fig. 4).

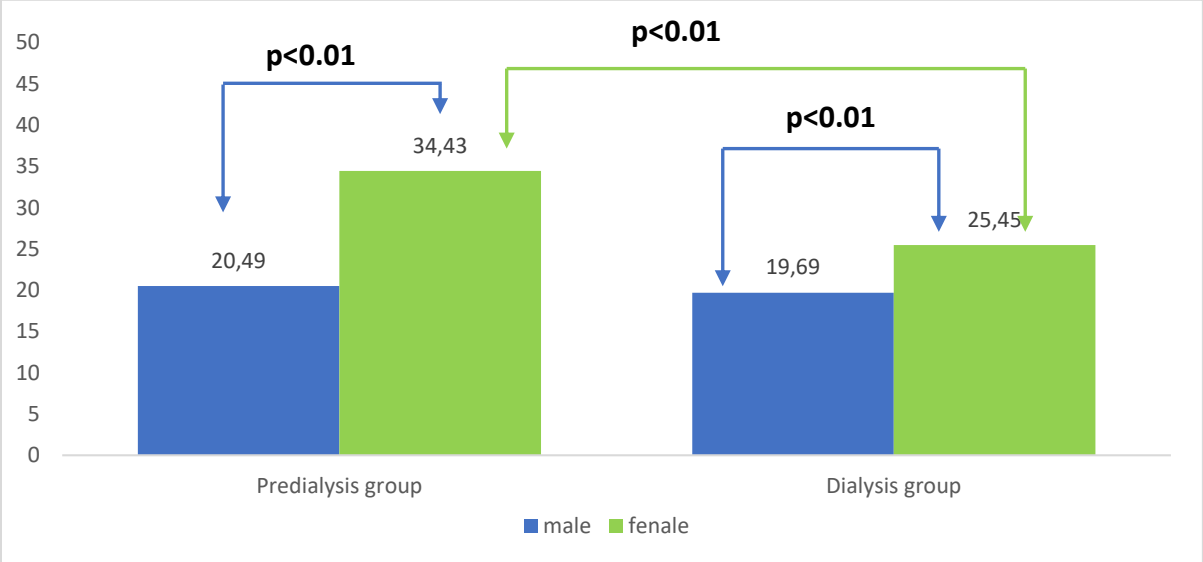

FIG. 4. Comparative analysis of the mean values of Visfatin according to the studied group and sex

In Figure 5 presents a comparative analysis between mean age, Visfatin threshold, gender, and study group, with significant differences (p <0.05). The predialysis stage in men is

associated with Visfatin levels above 16.92 ng / ml and older (66.71 years at 61.14 years), while in women it is associated with Visfatin levels above 16.92 ng / ml and younger. 63.3 years to 72.0 years). On the other hand, the dialysis stage in men is associated with Visfatin levels below 16.92 ng / ml and older (66.3 years to 54.3 years), while in women it is associated with Visfatin levels below 16.92 ng / ml and higher. low age (58.9 years to 69.1 years).

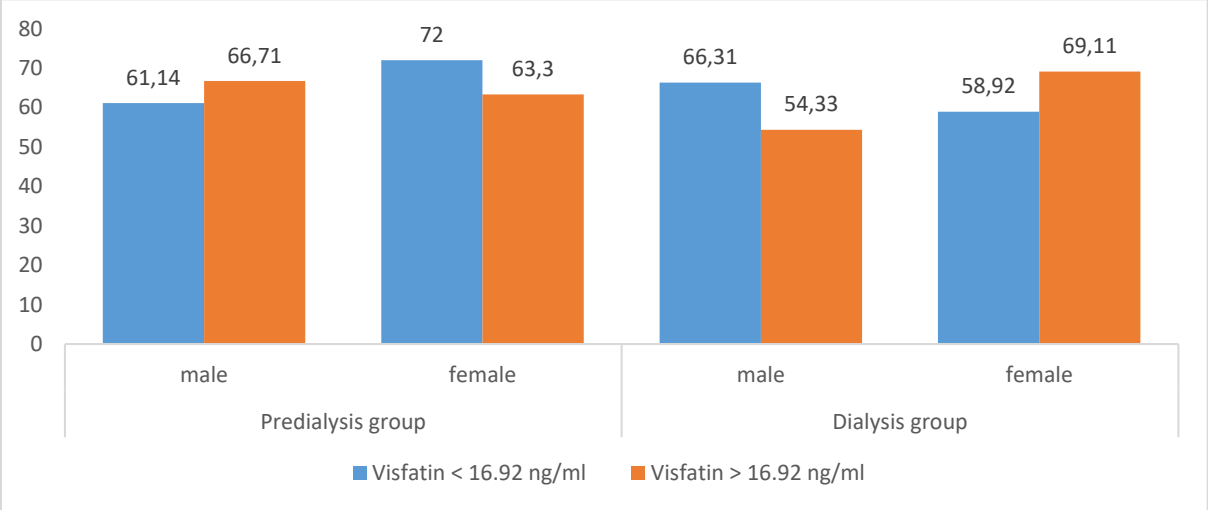

FIG. 5. Mean age of patients studied by sex, study group and Visfatin levels

Patients with hypertensive nephropathy were found to have significantly higher Visfatin values than the other two leading causes of CKD (diabetic nephropathy and chronic glomerulonephritis) (p = 0.031) (Fig. 6).

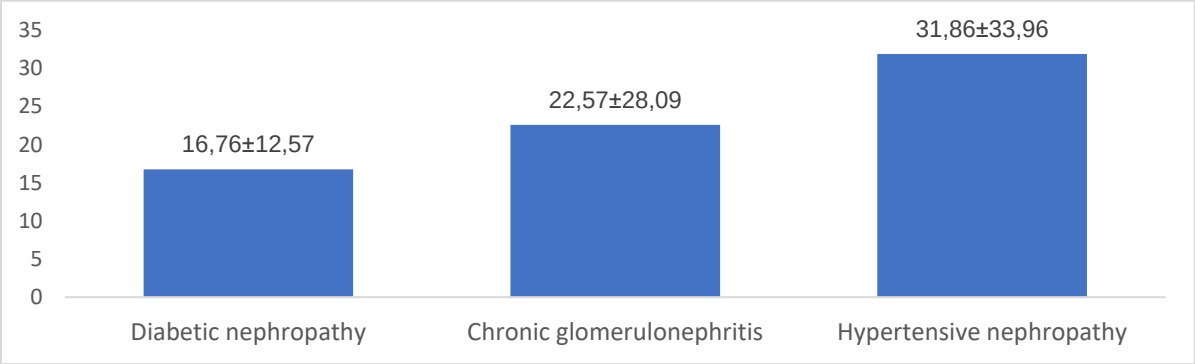

FIG. 6. Mean values of Visfatin according to the leading causes of CKD

Analysis of Visfatin levels by sex and the leading cause of CKD showed a significant difference between chronic glomerulonephritis (p <0.01) and hypertensive nephropathy (p <0.01), where marker levels were significantly higher in women than in men (Fig. 7).

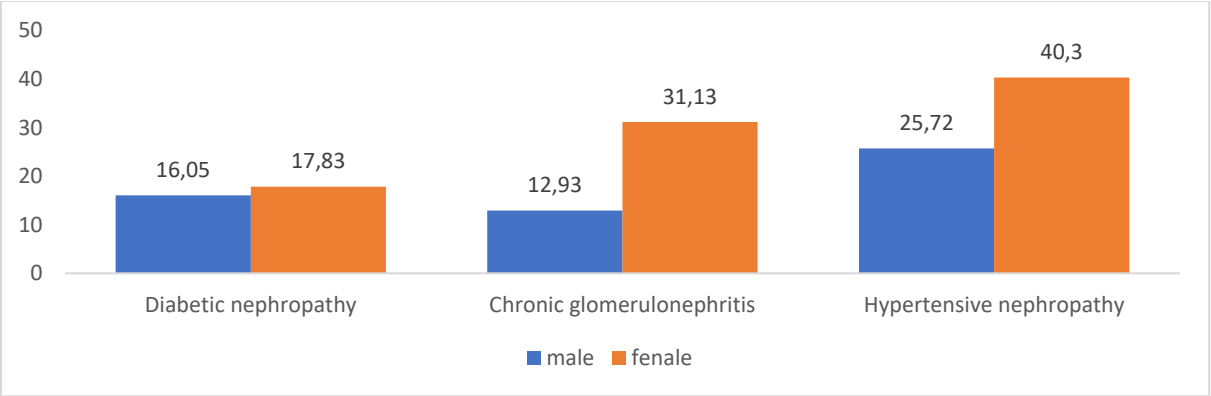

FIG. 7. Mean Visfatin levels by sex and leading cause of CKD

No association was found between serum levels of Visfatin, urea and creatinine, nor a difference in mean values according to the marker threshold.

On the other hand, serum Visfatin levels were found to correlate moderately in direct proportion to CCr in the group of predialysis patients (r = 0.476; p = 0.012) (Fig. 8). In 22.7% of cases, changes in CCr values were also associated with changes in Visfatin levels in patients with the predialysis group.

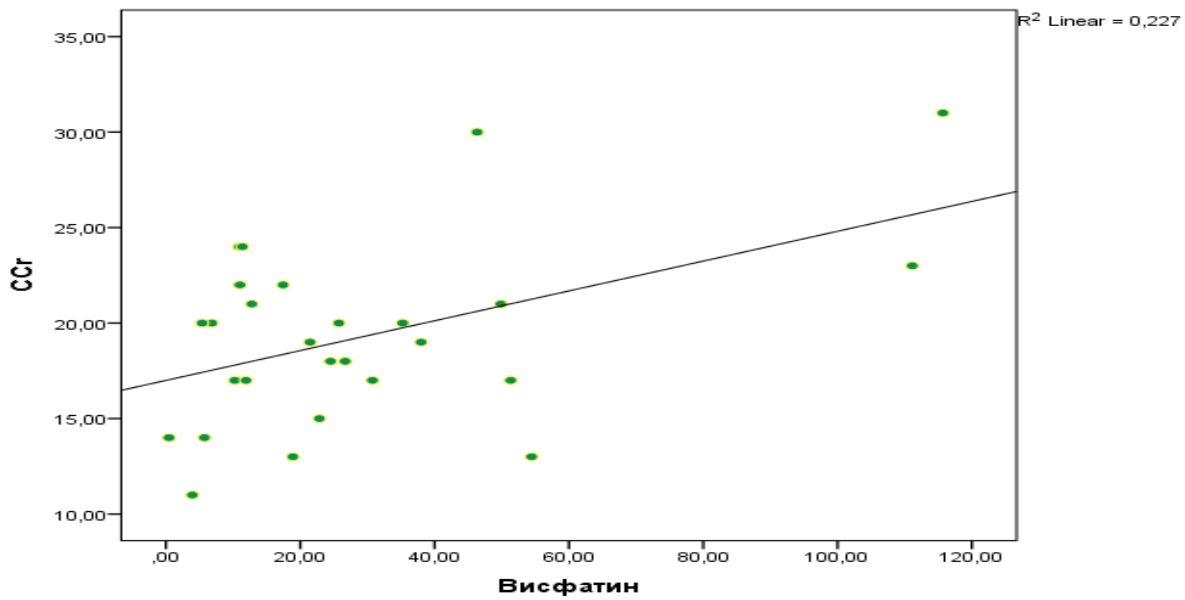

FIG. 8. Correlation analysis between serum levels of Visfatin and CCr

A significant difference was found in Visfatin levels according to the lower serum iron reference limit (p = 0.008), where low serum iron levels were associated with higher Visfatin levels (Fig. 9).

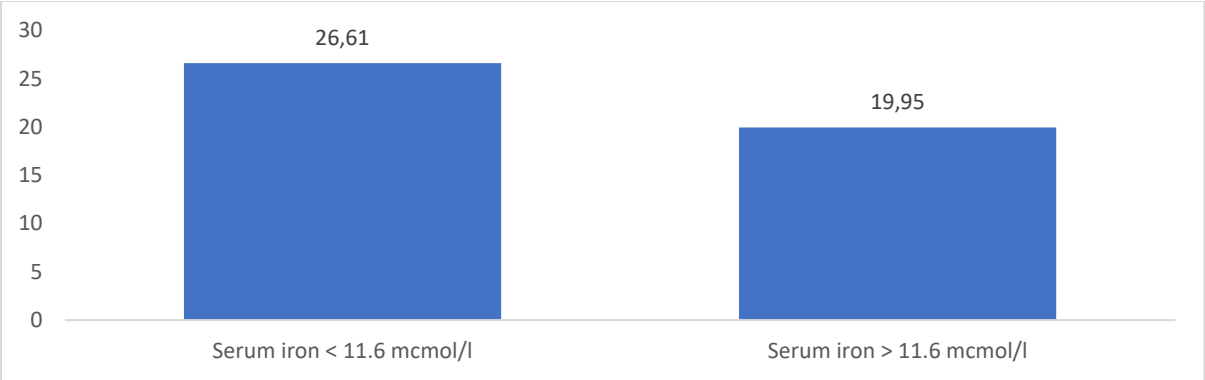

FIG. 9. Mean levels of Visfatin according to the serum iron reference limit

4.2. Assessment of the practical significance of the modern non-invasive biomarker (Visfatin), sensitive to the inflammatory process

In assessing the practical significance of Visfatin for the inflammatory process, the results were compared with data on CRP, uric acid and serum albumin levels..

Table 2. Characteristics of patients

Indicator		Predialysis group (n=30)	Dialysis group (n=50)	P value
CRP	mean±SD (range)	13.59±10.74 (0.13-38.80)	26.67±13.16 (1.22-57.06)	<0.001
Uric acid	mean±SD (range)	430.00±182.03 (156.0-926.0)	446.64±149.67 (135.0-904.0)	>0.05
Serum albumin	mean±SD (range)	38.81±6.56 (19.80-48.00)	34.82±4.97 (22.00-48.50)	0.003

Significant differences in CRP and albumin levels were found between the two groups of patients studied. There was no significant difference in uric acid levels.

On the other hand, a study of Visfatin levels according to uric acid reference values for men and women showed a significant difference between the sexes (p = 0.008) (Fig. 10).

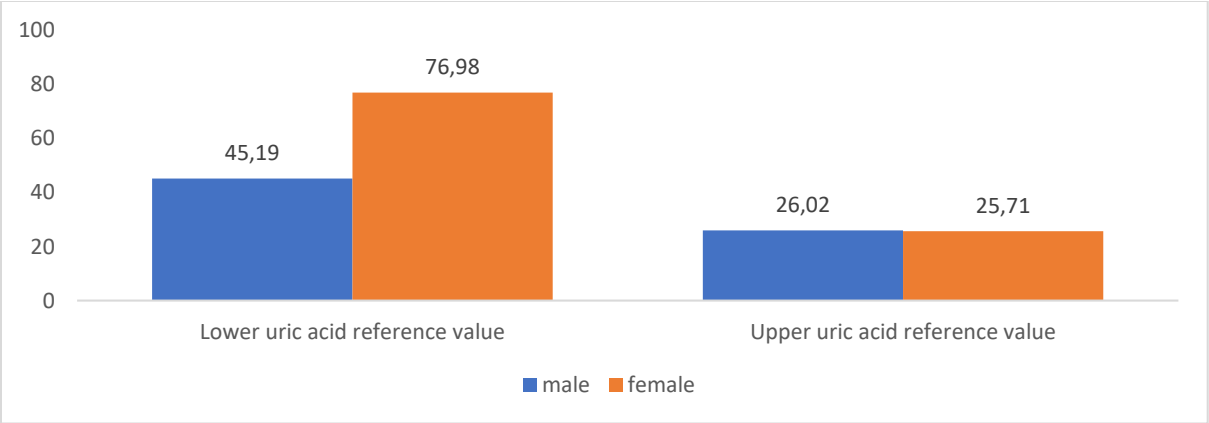

FIG. 10. Mean Visfatin levels according to uric acid and sex reference values

These results support the claim that low Visfatin levels are associated with elevated uric acid levels as a marker of the inflammatory process.

In the present study, 9 patients had normal CRP levels, and Visfatin levels in these patients were significantly lower than those with chronic inflammatory status (p = 0.01) (Fig. 11).

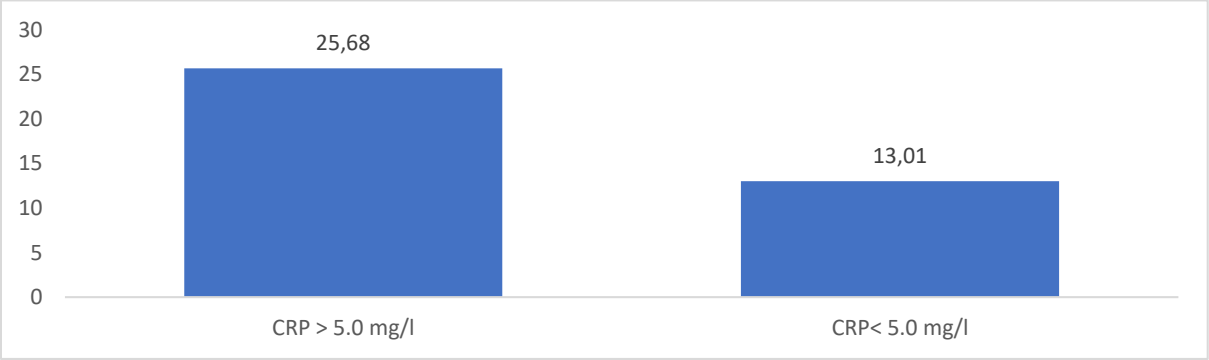

FIG. 11. Mean values of Visfatin according to CRP levels

High CRP levels correlated moderately with the progression of CKD and the inflammatory process (r = 0.462; p <0.001).

Low albumin levels correlated moderately with the progression of kidney disease (r = -0.329; p = 0.003).

The study of the relationship between serum levels of Visfatin and albumin showed the presence of moderate positive dependence in the predialysis group (r = 0.305; p <0.01) (Fig. 12). Analysis of Visfatin levels according to the upper (48.0 g / l) and lower (32.0 g / l) reference

values showed that low albumin levels were associated with low Visfatin levels and high albumin was associated with high Visfatin levels. (Fig. 13).

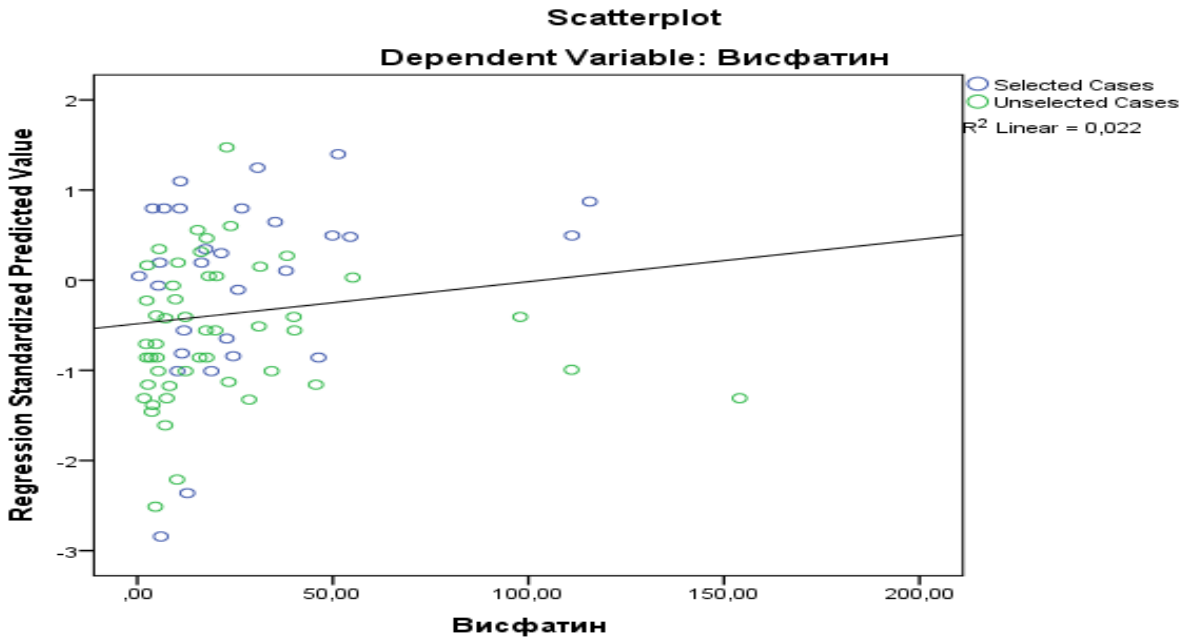

FIG. 12. Correlation analysis between serum levels of Visfatin and albumin in the predialysis group

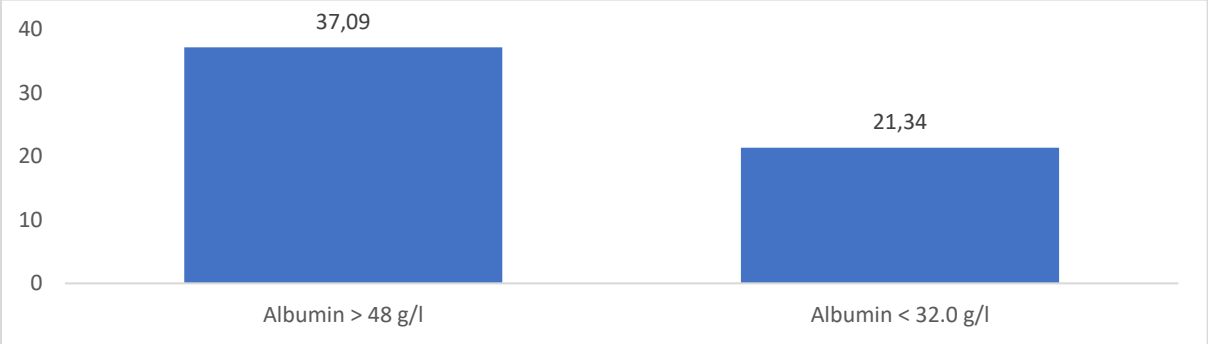

FIG. 13. Mean values of Visfatin according to albumin reference limits

There was also a significant difference in the mean values of albumin according to the determined threshold value of Visfatin (respectively 34.67 ± 6.21 to 37.98 ± 5.19) (p = 0.013) (Fig. 14), and this trend is maintained for the studied groups.

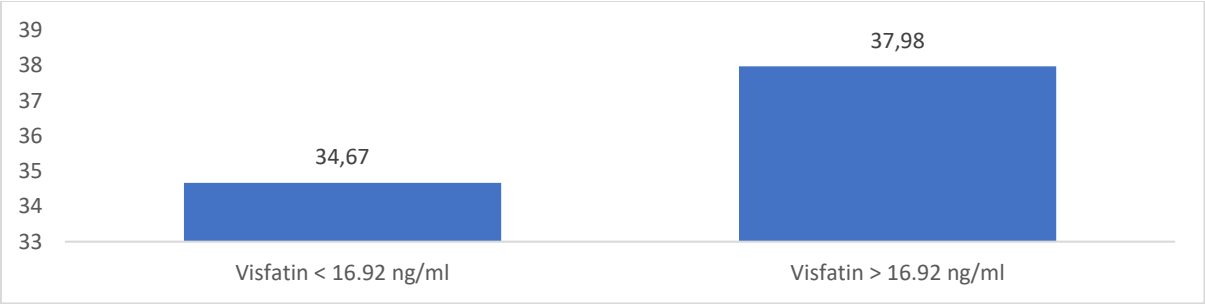

FIG. 14. Comparative analysis of serum albumin levels according to the Visfatin threshold

No association was found between uric acid and Visfatin levels, and the results showed the same uric acid values against the marker threshold (436.27 for values <16.92 ng / ml and 436.41 for values> 16.92 ng / ml, respectively).

There was a significant difference in albumin levels according to Visfatin levels between the group of patients in the pre-dialysis and dialysis stages (p <0.05) (Fig. 15).

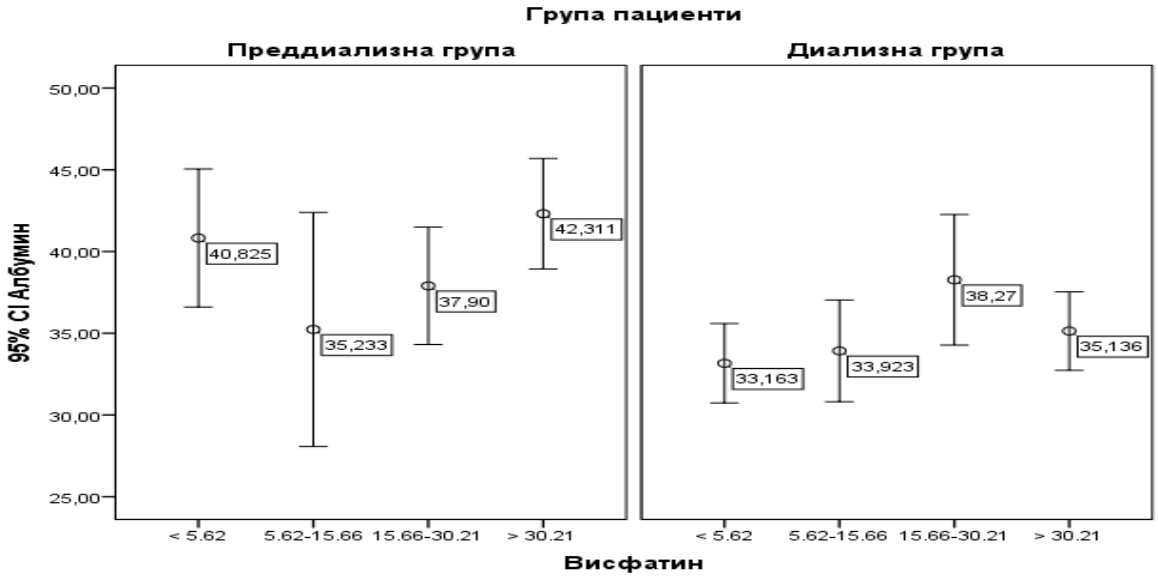

FIG. 15. Mean values of albumin according to Visfatin levels in the study groups

The study of the relationship between CRP and Visfatin levels found that the two markers correlated inversely in the group of patients receiving dialysis (r = -0.398; p = 0.001) (Fig. 16).

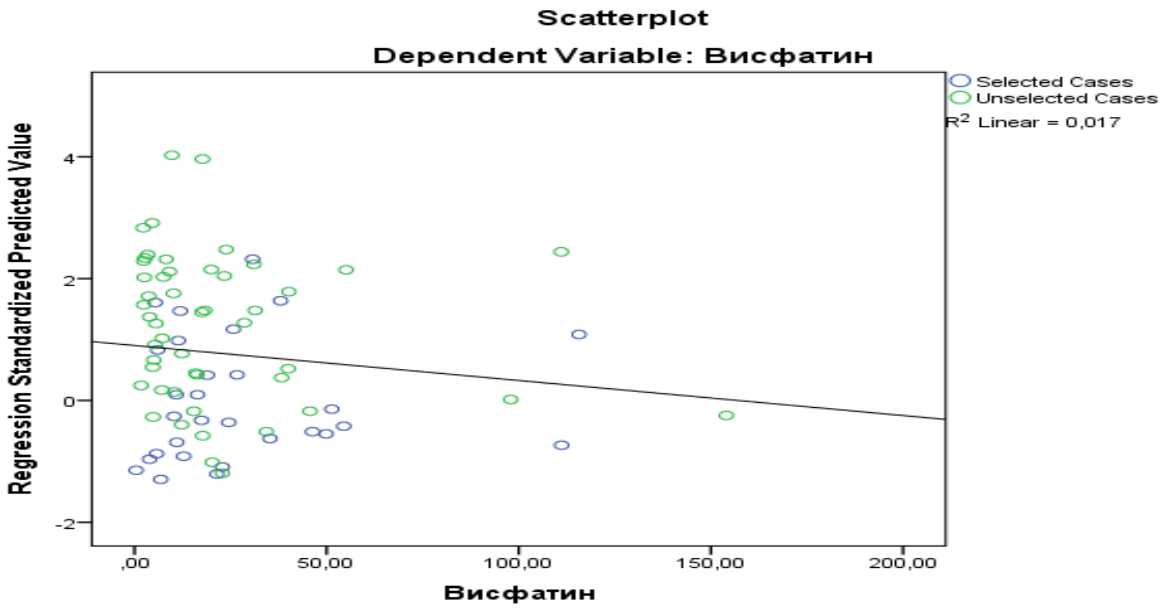

FIG. 16. Correlation analysis between serum levels of Visfatin and CRP in the dialysis group

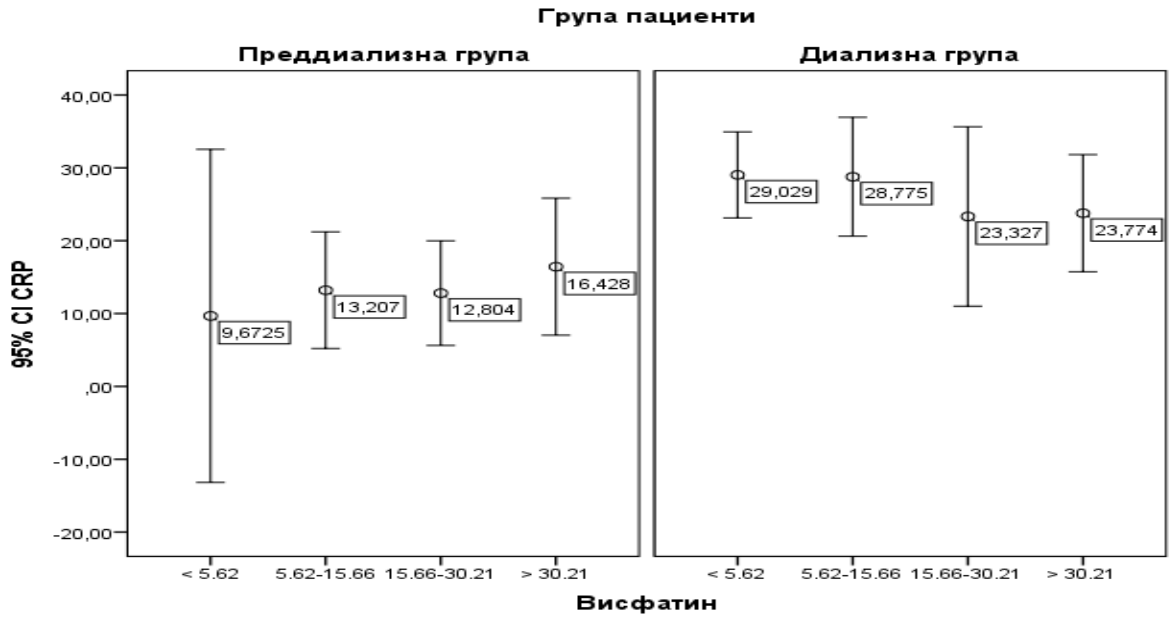

FIG. 17. Mean CRP values according to Visfatin levels in the study groups

There was a significant difference in CRP levels according to Visfatin levels between the group of patients in the pre-dialysis and dialysis stages ($p < 0.05$) (Fig. 17).

According to the results obtained, it can be said that low levels of Visfatin correlate inversely with serum CRP levels and in direct proportion to albumin levels in the progression of CKD.

4.3. Correlation between Visfatin and nutritional status of patients with chronic kidney disease

The assessment of the nutritional status of the studied patients with CKD was performed by examining BMI, folic acid levels and levels of Vitamin B12, and the results are presented in table. 3.

A significant difference in the values of the indicators in the two groups was found only in terms of BMI and folic acid, while the levels of Vitamin B12 did not differ significantly despite the lower values observed in patients in the dialysis group.

Table 3. Characteristics of patients

Indicator		Predialysis group (n=30)	Dialysis group (n=50)	P value
BMI	mean±SD (range)	26.76±3.69 (21.0-35.0)	18.06±1.11 (16.0-21.0)	<0.001
Folic acid	mean±SD (range)	15.71±8.59 (2.09-30.00)	19.54±8.71 (2.10-30.00)	0.05
Vitamin B12	mean±SD (range)	689.17±633.13 (210.0-3542.0)	567.76±254.15 (226.00-1975.00)	>0.05

There was no relationship between serum levels of Visfatin and BMI in patients in the study groups, but a significant difference in BMI values according to individual levels of Visfatin between the two groups of patients ($p < 0.05$) (Fig. 18).

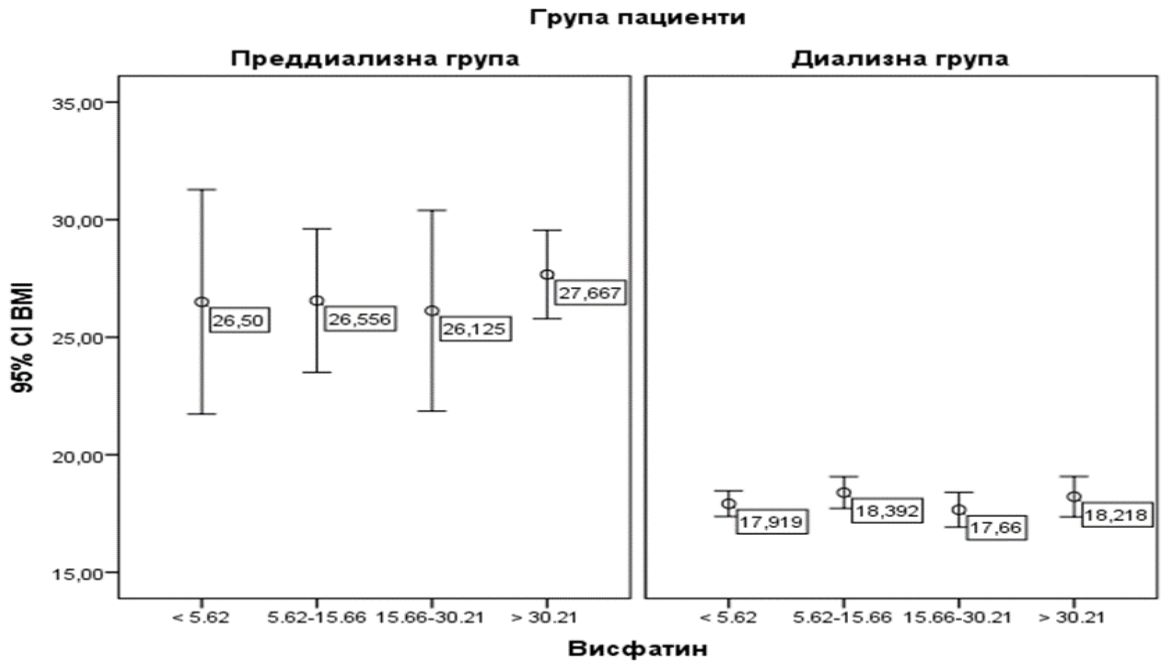

FIG. 18. Mean BMI values according to Visfatin levels in the studied groups

In the current study, only 5 patients (6.25%) with folic acid deficiency were characterized by lower levels of Visfatin (Fig. 19).

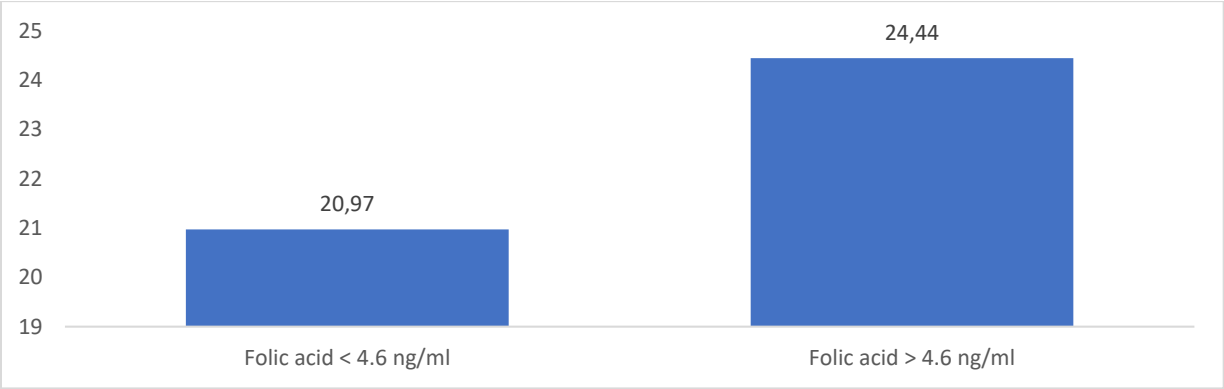

FIG. 19. Mean Visfatin levels according to the lower folic acid reference limit

A significant difference was also found in terms of folic acid levels and Visfatin levels ($p < 0.05$) (Fig. 20). It is impressive that folic acid levels are higher in patients in the dialysis group, which is a result of the therapy.

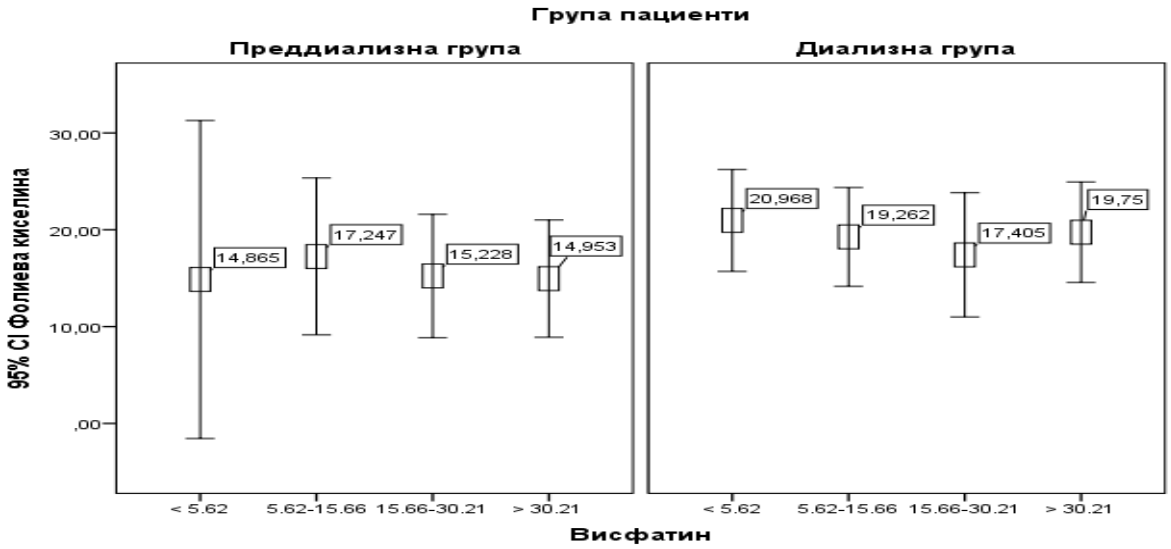

FIG. 20. Mean values of folic acid according to the levels of Visfatin in the studied groups

In the present study, there are no patients with Vitamin B12 deficiency, but 31 patients (38.75%) have hypervitaminosis, and in these patients Visfatin levels are significantly lower (p = 0.048) (Fig. 21).

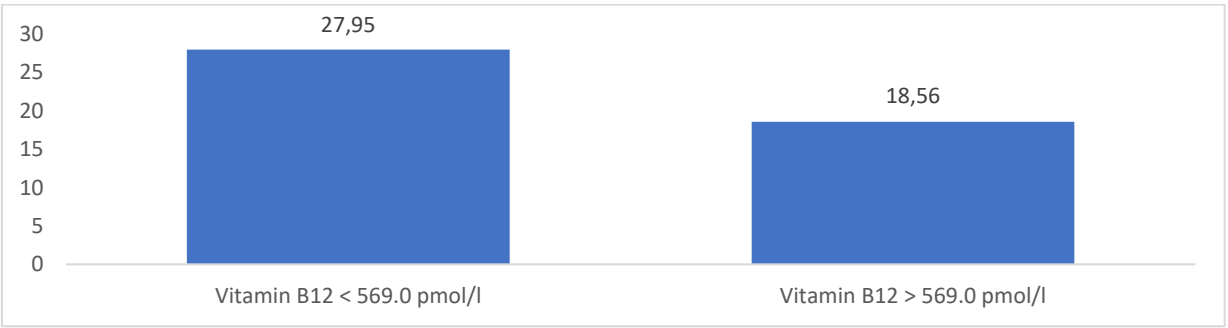

FIG. 21. Mean levels of Visfatin according to the upper reference limit of vitamin B12

The analysis of the change in vitamin B12 levels according to Visfatin levels in the two study groups showed that patients in the predialysis group showed a decreasing trend in Vitamin B12 levels with increasing Visfatin levels (p <0.05) (Fig. 22). On the other hand, with the progression of CKD to the terminal stage, approximately the same levels of Vitamin B12 are maintained, regardless of the levels of Visfatin, which is explained by the implementation of substitution therapy.

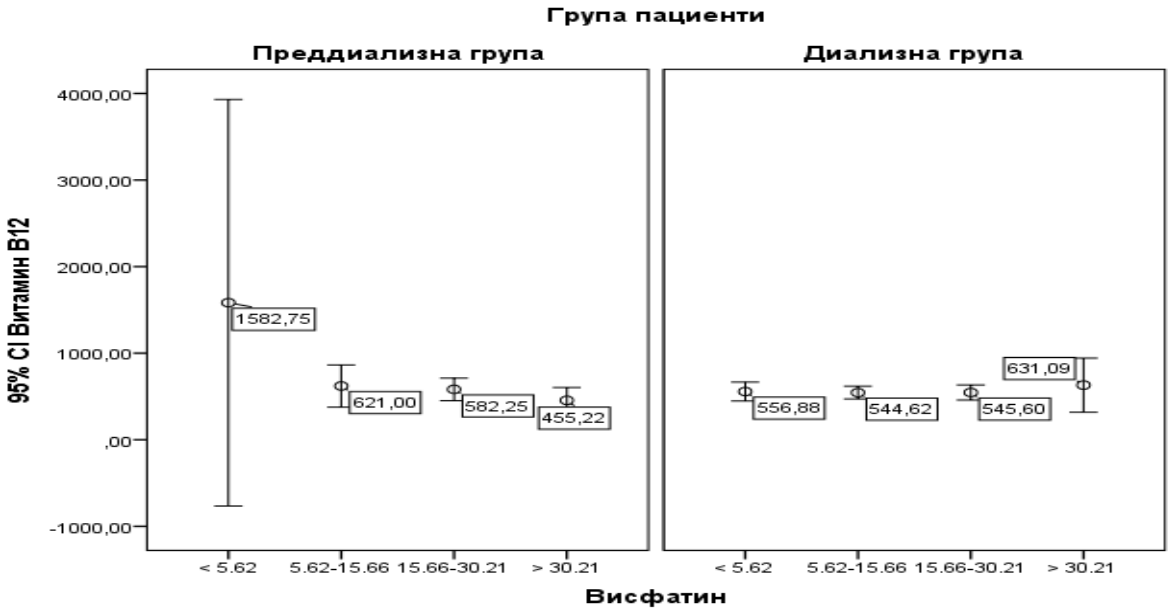

FIG. 22. Mean values of Vitamin B12 according to the levels of Visfatin in the studied groups

The analysis of the relationship between Visfatin levels and the nutritional status of the studied patients shows that there is a significant difference in the changes in the studied indicators in the two groups.

4.4. Comparison of the levels of the new non-invasive biomarker with indicators characterizing the inflammatory process in patients with chronic kidney disease

To assess the indicators characterizing the inflammatory process in patients with chronic kidney disease, sEPOR, iPTH and iFGF 23 were used, and the average values of the indicators in the two study groups are presented in Table. 4.

Table 4. Characteristics of patients

Indicator		Predialysis group (n=30)	Dialysis group (n=50)	P value
sEPOR	mean±SD (range)	211.47±378.85 (2.60-1676.50)	130.96±157.93 (35.40-970.80)	0.019
iPTH	mean±SD (range)	345.71±622.94 (77.0-1305.0)	622.94±735.48 (10.90-2500.00)	0.05
iFGF 23	mean±SD (range)	517.93±718.43 (12.88-2247.92)	1392.75±707.77 (166.44-2236.25)	< 0.001

There was a significant difference in the studied indicators between the two groups of patients, as iPTH and iFGF 23 have significantly higher values in those in the dialysis group, while sEPOR has higher values in patients in the predialysis group.

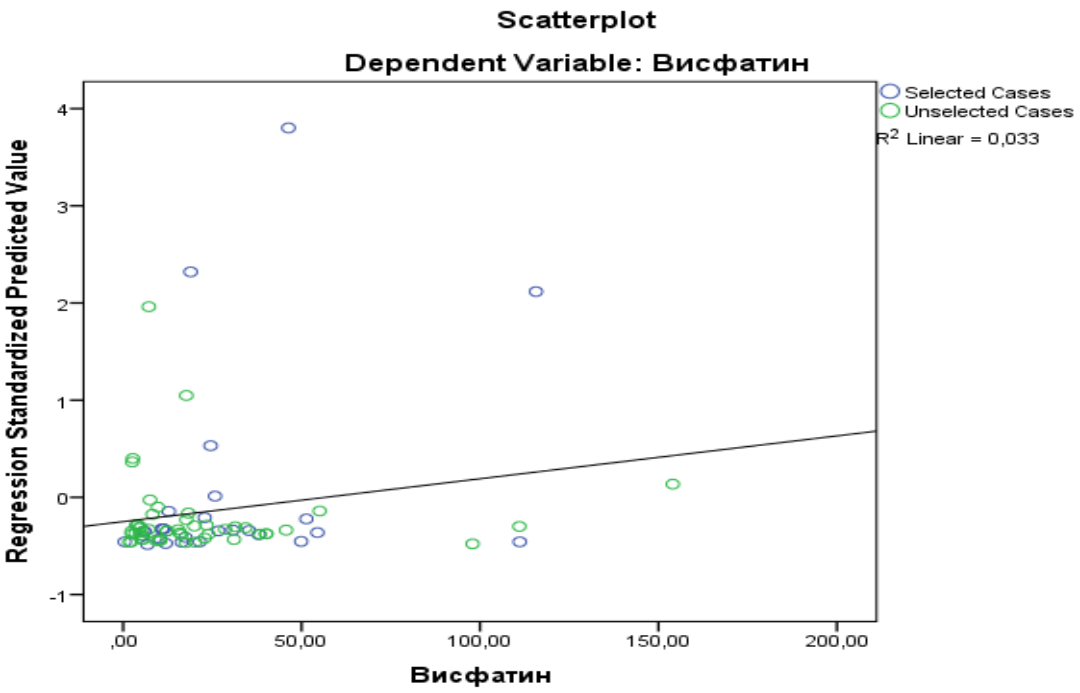

FIG. 23. Correlation analysis between serum levels of Visfatin and sEPOR in patients in the predialysis group

Analysis of the relationship between sEPOR and Visfatin shows that there is a moderate positive correlation between the two indicators (r = 0.336; p = 0.035) (Fig. 23), which shows that high levels of sEPOR are also associated with high levels of Visfatin in patients. in the predialysis group.

From the results of fig. 24 shows that there is a difference in the mean values of sEPOR according to the values of Visfatin, as in the pre-dialysis group the already proven positive association is presented, while in the dialysis group there is a peak of sEPOR at Visfatin levels between 5.62-15.66 ng / ml (171.38), after which it began to decrease significantly. These results demonstrate the association of sEPOR with the occurrence of anemic syndrome in patients with advanced CKD.

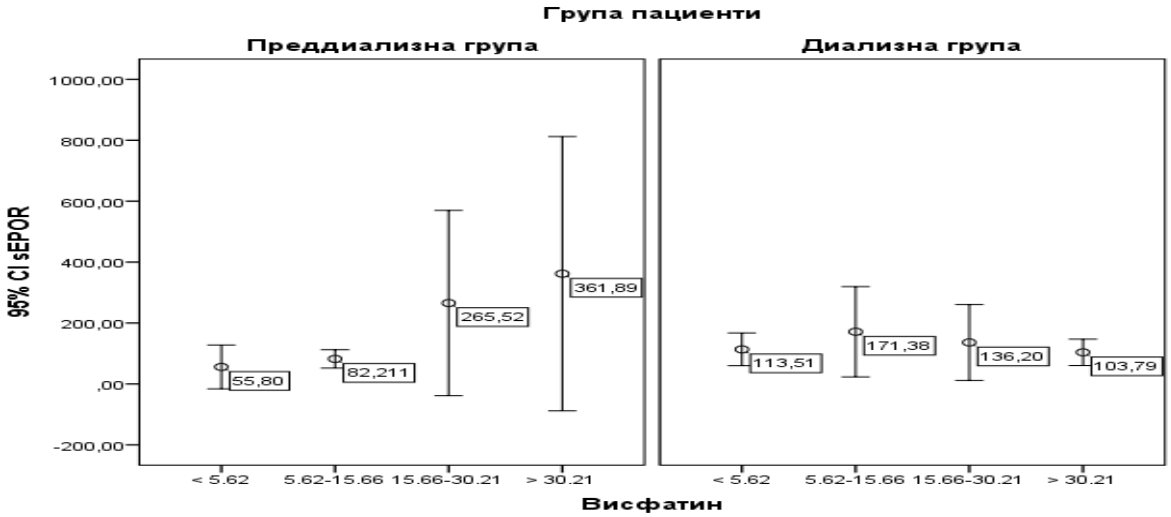

FIG. 24. Mean values of sEPOR according to Visfatin levels in the study groups

In the present study, only one patient had low levels of iPTH (10.90 pg / ml), which is characterized by extremely low levels of Visfatin (2.54 ng / ml), while individuals with iPTH values above the upper reference limit (90%) had significantly higher levels of Visfatin (23.96 ng / ml).

There was no relationship between iPTH and Visfatin values in either the predialysis or dialysis groups, but it can be said that there is a significant difference in iPTH levels from the Visfatin thresholds (p = 0.045), which show one and the same trend in both groups, but in the dialysis group the levels of iPTH are significantly higher (Fig. 25).

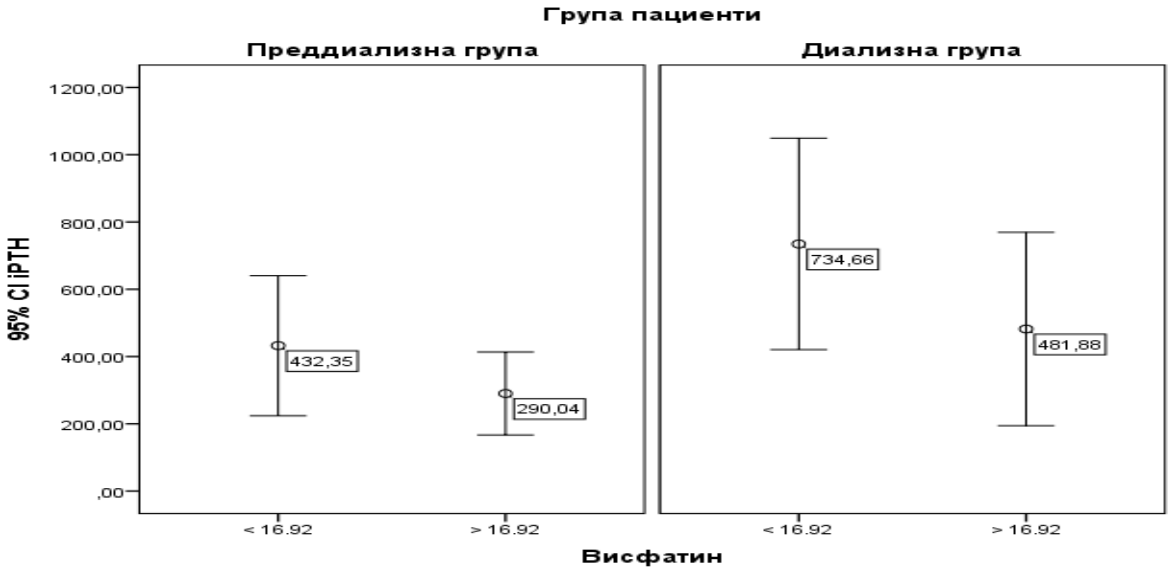

FIG. 25. Mean values of iPTH according to the threshold levels of Visfatin in the studied groups

There was a significant difference in the mean values of iFGF 23 according to the threshold value of Visfatin in patients in the predialysis group ($p < 0.05$), while in the dialysis group the levels of iFGF 23 remain consistently high regardless of the values of Visfatin (Fig. 26).

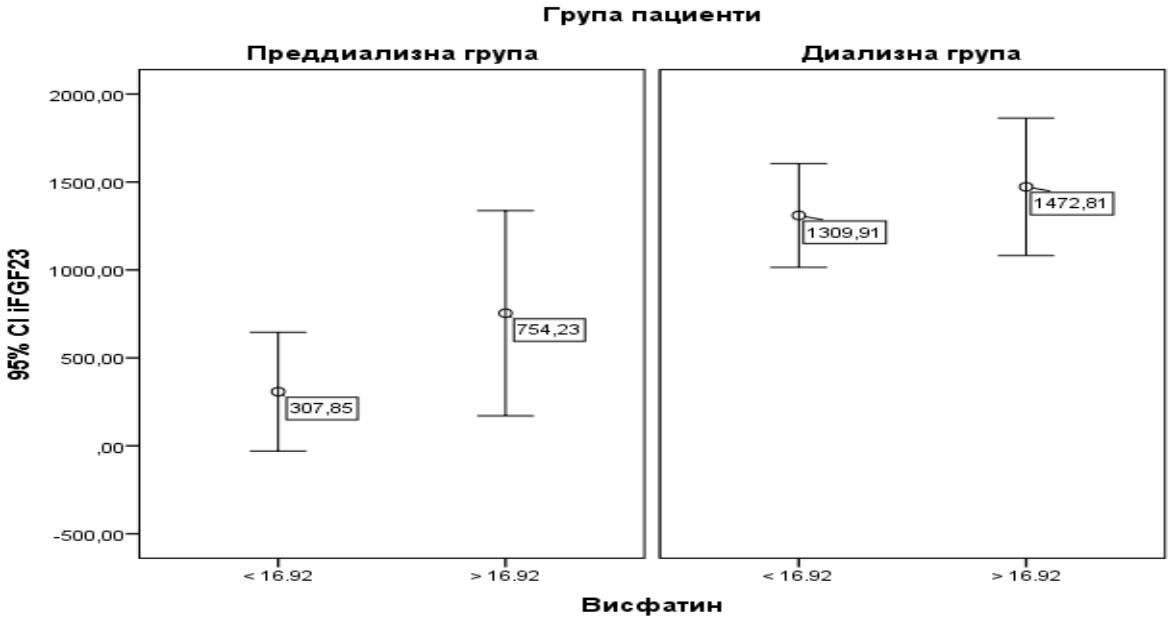

FIG. 26. Mean values of iFGF 23 according to the threshold levels of Visfatin in the studied groups

From the results obtained so far, the following main conclusions can be drawn regarding the low and high levels of Visfatin relative to the established threshold value (16.92 ng / ml).
Low levels of Visfatin are associated with diabetic nephropathy, high levels of CRP, high levels of uric acid and low CCr, the results of which are presented in Table. 5.

Table 5. Correlation analysis between low levels of Visfatin and some of the considered markers of the inflammatory process

Indicator	Correlation coefficient (r)	P value
Diabetic nephropathy	-0.328	0.036
CRP	-0.255	0.018
Uric acid	-0.323	0.039
CCr	0.682	0.021

High levels of Visfatin are associated with old age, women, high CCr and low serum iron levels and low levels of iFGF 23, the results of which are presented in Table. 6.

Table 6. Correlation analysis between low levels of Visfatin and some of the considered markers of the inflammatory process

Indicator	Correlation coefficient (r)	P value
Age	0.274	0.010
Gender - female	0.236	0.016
CCr	0.568	0.022
iFGF 23	-0.435	0.021
Serum iron	-0.233	0.015

4.5. Assessment of individual quality of life depending on nutritional status and the accompanying inflammatory process

Quality of life was studied in the 50 patients forming the group of dialysis patients.

Half of the patients underwent hemodialysis between one year and five years (Fig. 27). No significant difference was found according to gender and age.

From the point of view of BMI, there is no difference and dependence with the duration of hemodialysis, as BMI in patients undergoing hemodialysis under 1 year, the mean BMI is 18.3 ± 1.08 (17.40-19.50). In those who underwent hemodialysis treatment between 1 and 5 years, the mean BMI was 17.81 ± 1.01 (16.00-20.90), and in the group of patients with the longest duration of treatment BMI was 18.32 ± 1.21 (16.40-21.00).

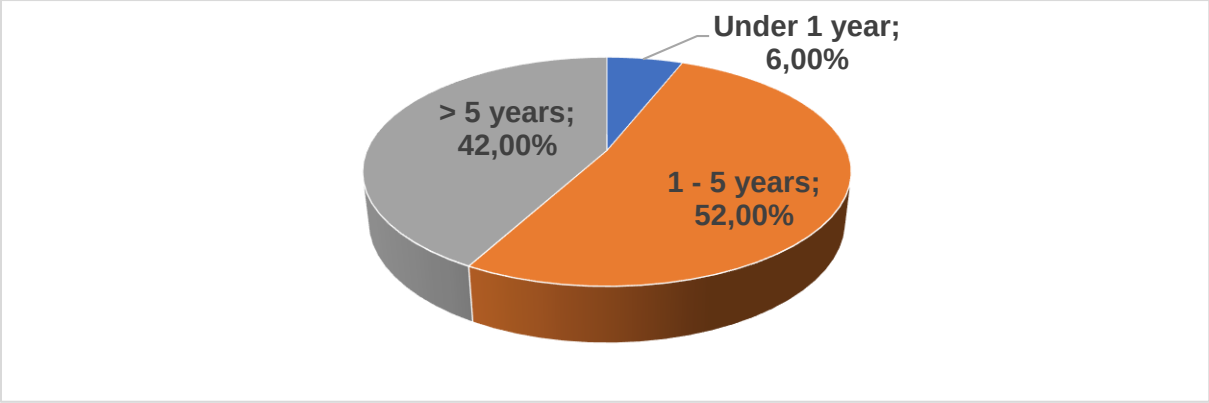

FIG. 27. Distribution according to the duration of hemodialysis treatment

No difference and dependence was observed with regard to the level of folic acid, as at the shortest duration of hemodialysis treatment the level of folic acid was 16.49 ± 5.54 (12.34-22.78). In the following periods, compensation of these low values and the level of folic acid was established (respectively 20.04 ± 8.84 in patients with a duration of 1-5 years and 19.36 ± 9.12 in patients with a duration of more than 5 years).

There is no difference and dependence with the duration of hemodialysis treatment with regard to the levels of Vitamin B12 and markers for the inflammatory process.

The analysis of Visfatin levels and the duration of hemodialysis treatment showed a significant difference ($p < 0.05$), which confirms the results so far that the levels of this marker decrease with the duration of dialysis treatment and the progression of CKD (Fig. 28).

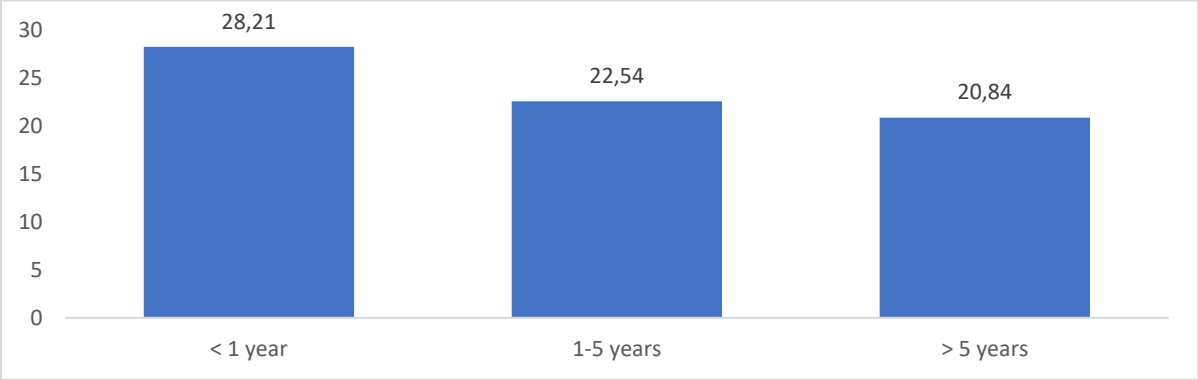

FIG. 28. Mean values of Visfatin according to the duration of hemodialysis treatment

About 3/4 (72.0%) of the patients indicated that they did not work, and there was no significant difference between the values of BMI, folic acid and Vitamin B12. No difference was found with regard to the markers of inflammation with the exception of albumin, where in patients who do not work the values are reduced ($p = 0.040$) (Fig. 29). Weak, moderate to inverse proportional relationship between albumin levels and performance was observed in the studied patients ($r = -0.292$; $p < 0.05$).

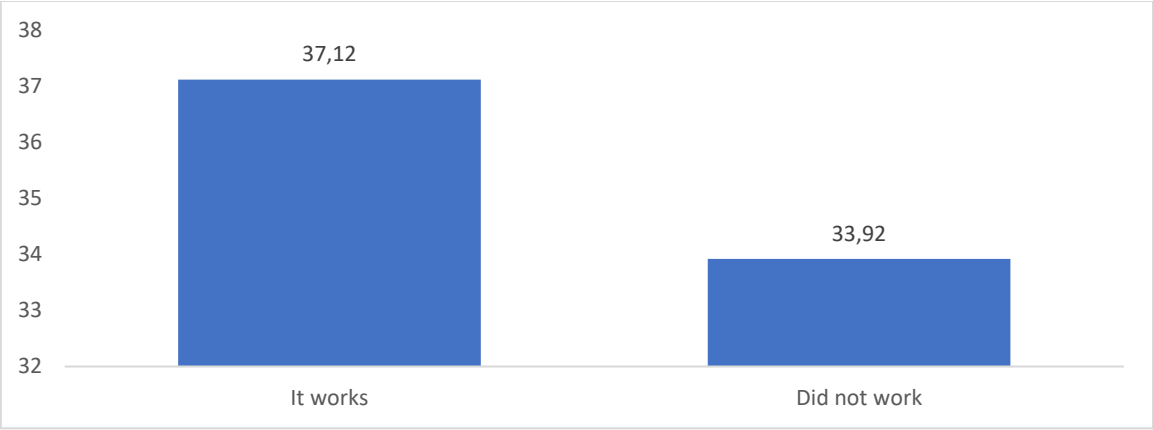

FIG. 29. Average values of albumin according to the working capacity of the patients

Over 1/3 (40.00%) of the patients reported feeling pain in the last four weeks, with no significant difference and dependence between pain and BMI, folic acid levels, Vitamin B12 and albumin. There is no difference and dependence on pain with regard to markers of inflammation.

Low levels of Visfatin correlated with a lower assessment of patients' health (r = -0.399 p <0.05.) (Fig. 30).

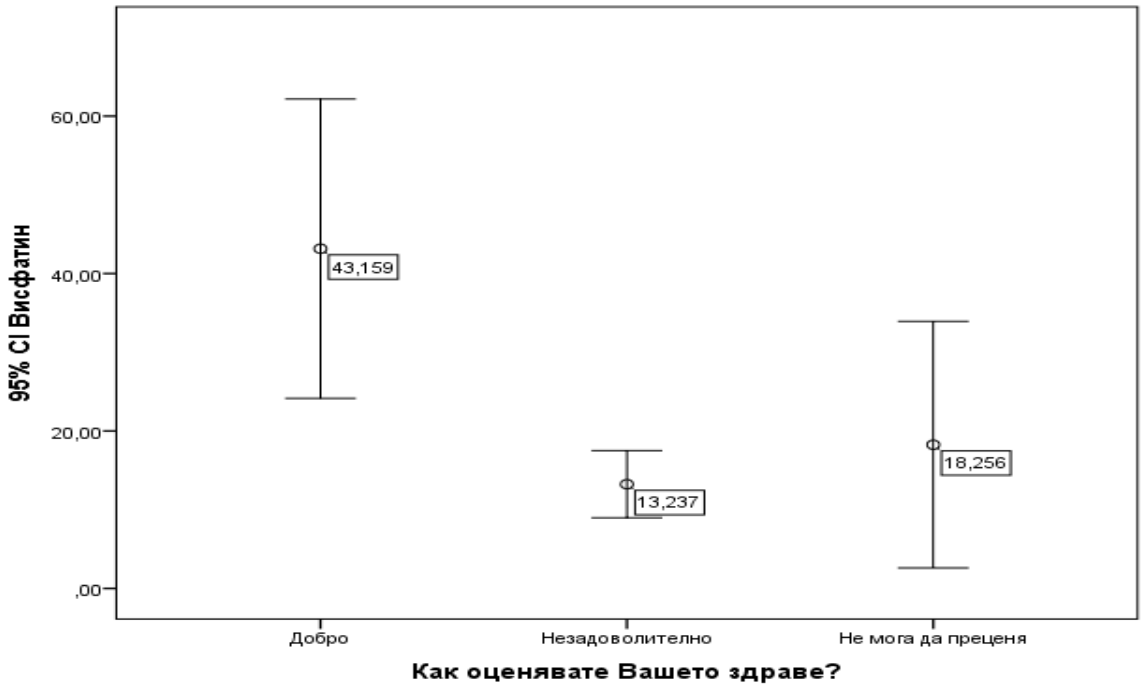

FIG. 30. Mean value of Visfatin according to health assessment

From the point of view of fatigue, it was found that patients who felt tired had significantly higher levels of Visfatin (Fig. 31) ($p < 0.01$). Like all patients, they reported feeling tired and had Visfatin levels above the threshold.

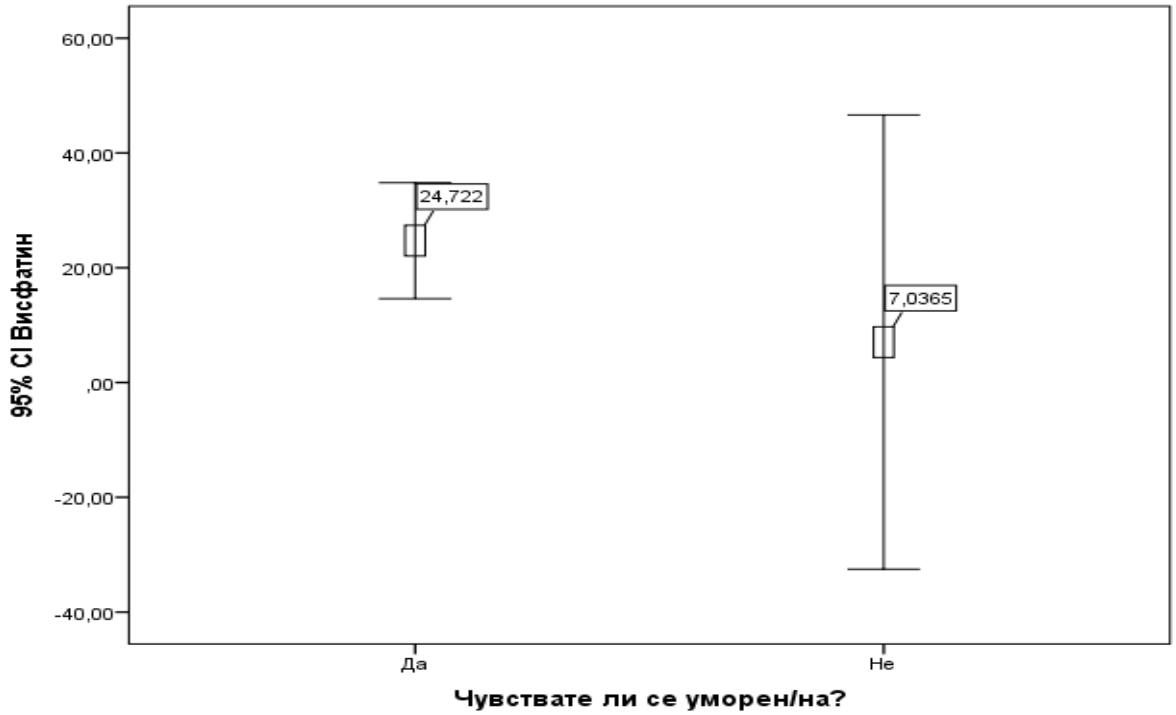

FIG. 31. Average value of Visfatin according to the presence of fatigue

Pain is associated with poorer quality of life, with 40.0% of dialysis patients in the current study experiencing pain in the last four weeks.

Analysis of the association of pain with Visfatin levels showed that patients who experienced pain had significantly lower Visfatin levels ($p = 0.013$), which further supports the data from the above studies that Visfatin levels decrease with the progression of CKD (Fig. 32).

On the other hand, despite the lack of significant difference, patients with pain were found to have higher CRP levels (26.54 ± 11.83 for pain patients to 25.91 ± 13.05 for pain-free patients, respectively).

High levels of CRP and low levels of Visfatin in patients with pain once again confirm the inverse relationship between the two markers, which was found earlier.

FIG. 32. Mean levels of Visfatin according to the presence of pain

In 30%, pain affected their performance, which further reduced their quality of life, with Visfatin levels in these patients being even lower (p = 0.009) (Fig. 33).

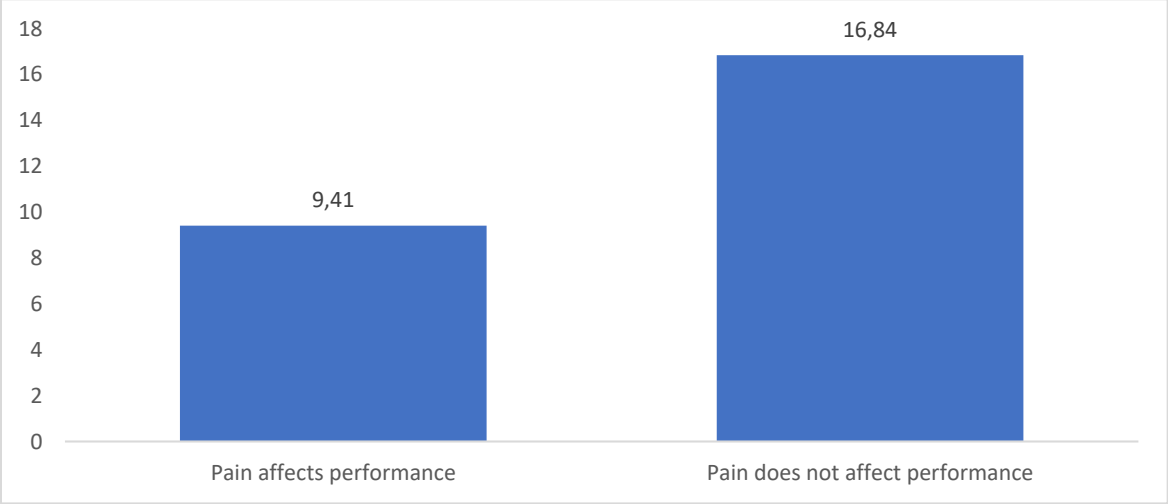

FIG. 33. Mean levels of Visfatin according to the effect of pain on performance

In terms of physical activity, 46.0% have light physical activity during the day and 54.0% moderate. The analysis of Visfatin levels according to physical activity showed that patients with light physical activity had lower Visfatin levels (p = 0.034) (Fig. 34). These results are also confirmed when Visfatin levels are analyzed through the prism of health and physical activity assessment (p <0.01). Both in patients with good health and in those with unsatisfactory light physical activity are associated with low levels of Visfatin (Fig. 35).

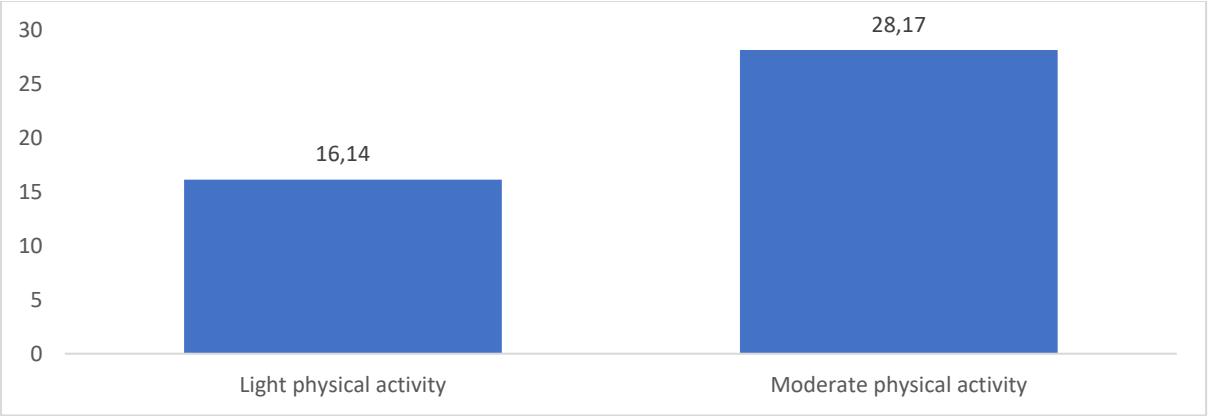

FIG. 34. Mean levels of Visfatin according to physical activity

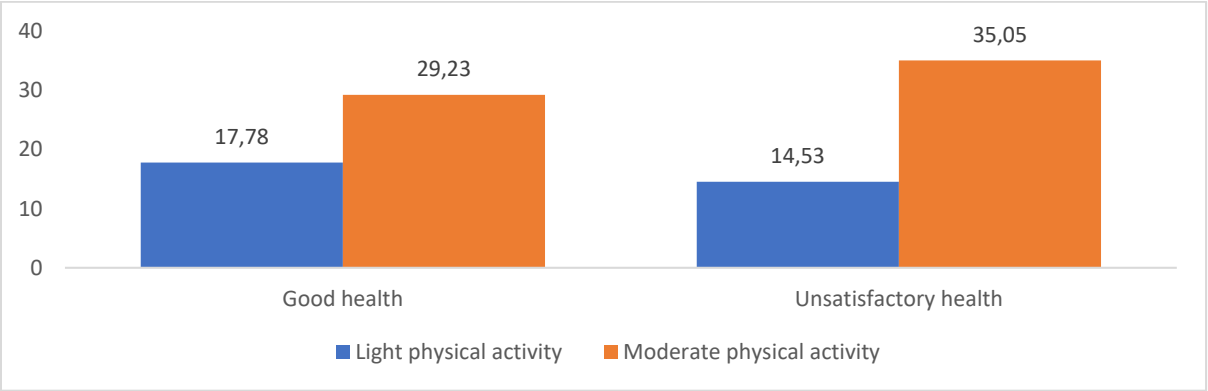

FIG. 35. Mean Visfatin levels as assessed by health and physical activity

Prior to initiating hemodialysis, 32.0% of patients were frequently ill, and analysis of Visfatin levels showed that these patients had significantly lower values than patients who had not been treated (p = 0.027) (Fig. 36).

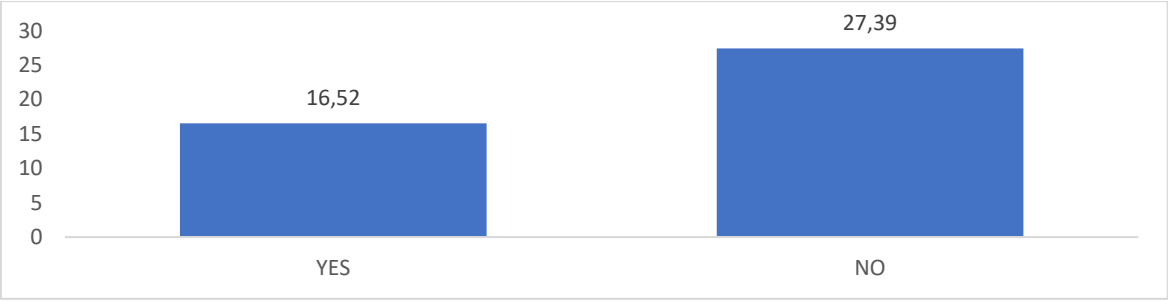

FIG. 36. Mean levels of Visfatin according to the common disease before starting hemodialysis treatment

From the point of view of health assessment, it was also found that frequent illness was associated with low levels of Visfatin ($p < 0.01$) (Fig. 37).

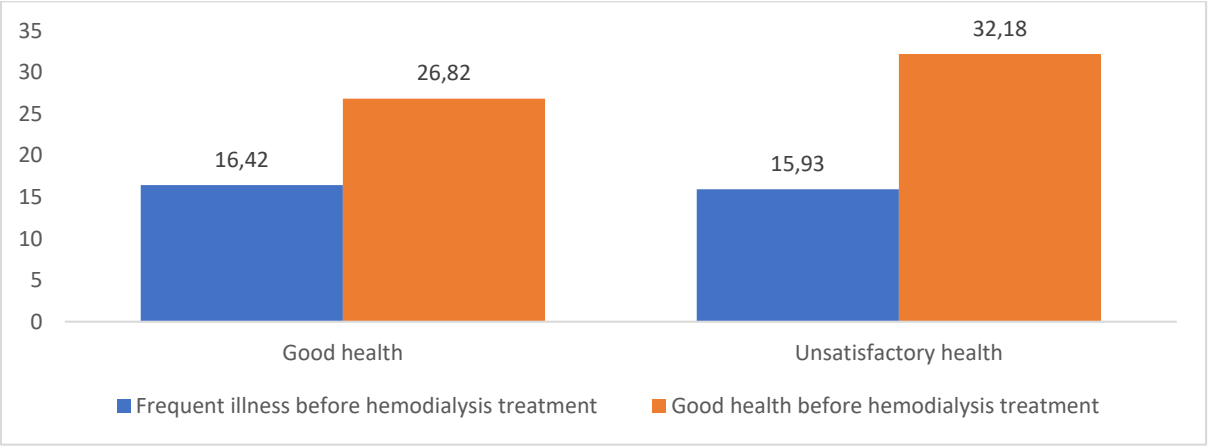

FIG. 37. Mean Visfatin levels as assessed by health and disease status prior to hemodialysis treatment

Frequent hospitalizations were reported in 16.0% of patients, with significantly lower levels of Visfatin ($p = 0.045$) in this group (Fig. 38).

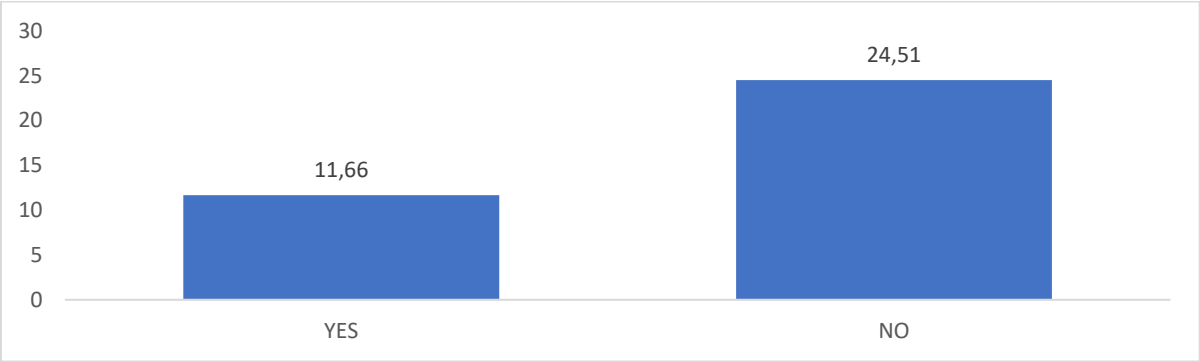

FIG. 38. Mean Visfatin levels according to frequent hospitalizations

Although no significant difference was found, Visfatin levels in the presence of stress were lower than in non-stressed patients (20.19 ± 23.75 in the presence of stress and 29.79 ± 40.84 in the absence of stress, respectively).

Patients' self-esteem is associated with a better quality of life, with 50% of patients in the current study describing their self-esteem as satisfactory (Fig. 39).

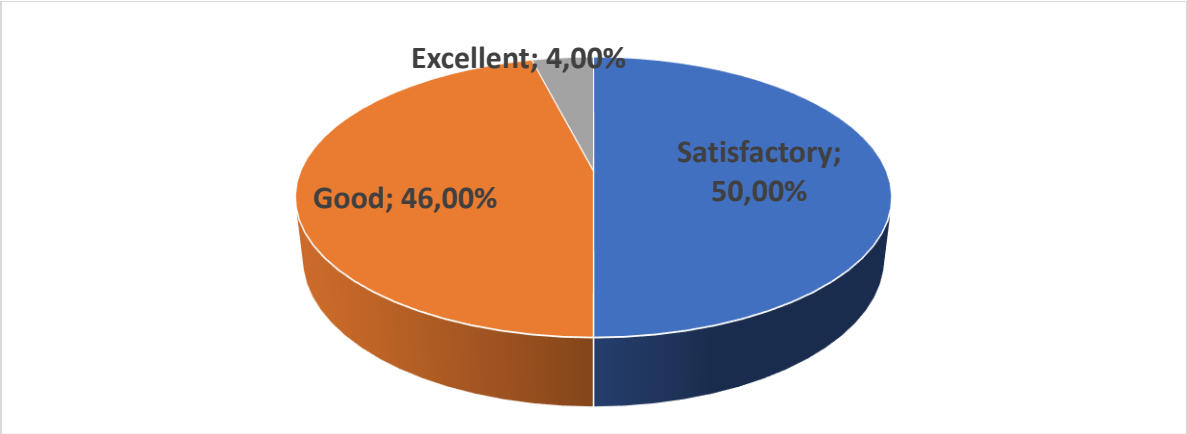

FIG. 39. Distribution of patients according to self-esteem during the interdialysis period

Although there is no direct relationship with Visfatin levels, their analysis once again confirms that high Visfatin levels are associated with better health and quality of life in the current sample (p = 0.026) (Fig. 40).

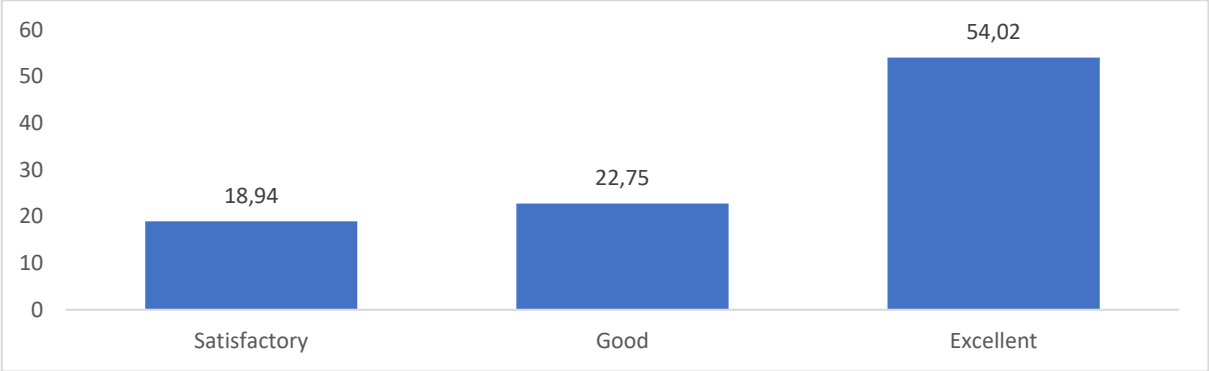

FIG. 40. Mean Visfatin levels according to self-esteem

Of the patients who took part in the survey, 96% reported light to moderate physical activity in their daily lives. The number of those who do not play sports prevails - 88%.

According to the indicator "difficulty in normal physical activity", the number of participants who supported this statement is 54%. Respondents report a sharp decrease in motor activity in their daily lives (<1 km) - 58%.

Of the patients who took part in the survey, the percentage of those who are unemployed is the highest - 72%. Only 28% say they are employed. 70.8% of working patients report the

presence of climate change at work after starting hemodialysis treatment. However, 66.7% of them say that this was well received by their boss. Only 15.2% felt tension at work. Of working patients, 36% share support from their colleagues in the workplace.

In terms of family / social environment, 94% of people received the support of their families after starting replacement therapy for kidney function. 66% of the respondents have kept their positive contacts in their social environment. Slightly more than half - 56%, share problems in their intimate life.

More than half (54%) of the patients surveyed said that their health improved and their complaints and feelings of pain decreased after starting hemodialysis treatment. 40% of people answered positively for the presence of a symptom of pain, and 46% of them said that it did not affect their ability to work. Slightly more than half of the respondents - 54% describe their health as unsatisfactory. However, only 42% of this group visit their GP / dentist regularly.

80% of the surveyed patients do not think that they need more frequent hospitalizations. Nearly 2/3 of the patients say that they do not get sick more often than before starting hemodialysis treatment. It is important to note the more frequent fatigue (82%), emotional tension (48%), tension (58%), stress (46%) and sleep problems (68%) in the studied patients undergoing replacement therapy for renal function. Just over 1/3 of the surveyed patients share a lack of self-confidence, and 50% of the other patients describe it as satisfactory.

According to 60% of patients in the dialysis group, the medical care provided to them by health professionals is good. 74% believe that the medical staff spends enough time for them, and 86% of respondents are satisfied with the care provided by the treatment team.

In the interviewed patients it is noticeable that with increasing the period of conduct (> 1 year) of dialysis treatment decreases their physical activity. There is also a small percentage of working patients who report climate change in their workplace after starting treatment. Most of them have the support of their employer and colleagues, but not a small number of those who share about the tension in the workplace and change in attitudes towards them.

Patients who report reduced physical activity, limited to missing social contacts, predominate. There is tension in their workplace and pronounced psycho-emotional stress. From everything so far it can be concluded that their quality of life would be reduced.

The fact that the surveyed participants have the support of their families is positive. There is a high percentage of those who report a reduction in complaints and pain that would lead to future hospitalizations after starting replacement therapy for kidney function.

It is clear from the respondents that dialysis patients do not visit their GPs often, thus not paying enough attention to their health and psycho-emotional problems.

V. DISCUSSION

In his study, Fathima el al. [47] reported that serum concentrations of visfatin were significantly elevated in patients with CKD compared with controls in healthy patients. Serum visfatin levels in the different stages of CKD were compared, with a progressive increase from stage 2 to stage 5 CKD. It is relatively increased in the early stages of CKD and continues with progression of renal dysfunction, inversely correlated with creatinine clearance (CCr). Studies to date have shown a strong positive correlation between serum visfatin and blood urea, serum creatinine, CRP, triglycerides (TGL) and VLDL-cholesterol. These findings are consistent with the study by Tang et al, who reported an increase in serum levels of visfatin in all stages of CKD. [163] The results obtained in the present dissertation do not support the statement described by the authors so far. Decreases in Visfatin levels with progression of CKD were observed in the study groups. No relationship was found between urea, serum creatinine and Visfatin. In 22.7% of cases, changes in CCr values were associated with changes in Visfatin values in the predialin group of patients.

In a study by Korzh et al. (2020) found that serum Visfatin was significantly and inversely bound to eGFR ($r = –0.79$, $p < 0.0001$) and positively bound to urinary albumin ($r = 0.71$, $p < 0.0001$). in study participants. [85]

CKD is a condition of chronic persistent low-grade subclinical inflammation in which there is a chronic systemic increase in proinflammatory mediators and cytokines released from adipose tissue. The kidneys play an important role in the excretion of adipokines. Decreased renal function in patients with CKD leads to altered use of these adipokines, causing them to accumulate in the body. Therefore, serum levels of visfatin are elevated in patients with CKD, which plays an important role in innate immunity. It is also secreted by activated lymphocytes, monocytes and neutrophils and stimulates the secretion of IL-6 by P38 mitogen-activated protein kinase (MAPK) and MAPK kinase-1 pathways. [10, 112, 122] Visfatin also induces the expression of inflammatory mediators in endothelial cells through the nuclear factor (NF) –κB pathway. Therefore, it may also play an indirect role in cardiovascular disease (CVD) in patients with CKD.

Dyslipidemia, which is an atherogenic risk factor, contributes to the initiation and progression of CKD in part by stimulating and enhancing the effect of inflammatory mechanisms. In the study by Fathima el al. [47] significantly higher levels of serum triglycerides (TG) and VLDL were observed in the studied patients compared to controls. The

researchers also observed significantly lower levels of serum HDL compared to the control group of healthy patients and no significant difference in TG and LDL in the two groups. The study demonstrated a significant positive correlation between serum levels of visfatin with TG and LDL, which is an independent strong predictor of cardiovascular events. Compared with the obtained HDL values, a negative correlation is observed, which is considered to be an independent strong opposite predictor of CVD.

Accumulated evidence suggests that adipose tissue is no longer considered only a depot for triglyceride storage, but also a true endocrine organ that synthesizes and secretes a wide range of diverse bioactive factors called adipokines. These adipokines can be secreted and act locally in adipose tissue and have been shown to play a variety of roles in vivo, including modulation of lipid metabolism, inflammation, insulin resistance, immune stress response, and vascular homeostasis. [154] Visfatin was initially identified as a novel adipokine with insulin-mimetic properties in mice and is an adipocytokine whose circulating concentration has been found to be elevated in metabolic disorders and obesity. [154] Adipocytes are the main source of visfatin and the calculated physiological plasma level of visfatin is 15 ng / ml, with plasma concentrations of visfatin increasing with fat accumulation. [105, 152, 154] Several clinical studies have shown that circulating concentrations of Visfatin are significantly increased in patients with CKD compared to normal controls. [13, 176]

This statement is also supported by our results. Persistently elevated levels of Visfatin have been reported in both pre-dialysis and dialysis patients.

In another study by Ayar et al. In 2014, Visfatin levels were found to be lower in dialysis patients than in the non-dialysis group (31.9 ng / ml for dialysis patients and 33.1 ng / ml for non-dialysis patients, respectively).) [14]. These results are also supported in the present study where mean levels of Visfatin in the dialysis group were significantly lower (21.7 ng / ml for the dialysis group and 26.7 ng / ml for the pre-dialysis group, respectively).

In patients with diabetes, there is a significant negative correlation between Visfatin plasma concentrations and creatinine clearance or endothelial function as assessed by FMD. [162] In this prospective group, we first show that circulating visfatin is an independent predictor of eGFR reduction.

Looking for a potential link between CRP and whistfatin, the researchers looked at significantly higher levels of CRP in patients with CKD than the control group. This proves the positive potential link between inflammation and Visfatina. Inflammation and dyslipidemia are

well known as risk factors for atherosclerosis. Visfatin plays an important role in linking inflammation and lipid dysregulation with atherosclerosis.

In the present study, a significant difference in CRP and albumin levels was found between the two groups of patients studied, given the presence of inflammatory status. High CRP levels correlated moderately with the progression of CKD and the inflammatory process ($r = 0.462$; $p < 0.001$). In the study group of dialysis patients, CRP correlated inversely with Visfatin values. On the other hand, in the absence of an inflammatory process (CRP <5.0 mg / l), Visfatin levels were found to be significantly lower (13.01 ng / ml versus 25.68 ng / ml), thus supporting the claims in the literature that lack of inflammation Visfatin levels are lowered.

Studies to date have shown that elevated levels of visfatin can be considered as a marker of endothelial dysfunction (ED) and prediction of the incidence of cardiovascular disease in patients with CKD. Kim et al. observed the effect of visfatin on vascular endothelium, which induces the appearance of inflammatory mediators in cultured human endothelial cells via the NF-κB pathway. [83] This biomarker belongs to a family of medium molecular weight uremic retention substances that induce leukocyte adhesion to endothelial cells and endothelium by induction of cellular adhesion molecules such as intracellular adhesion molecule -1 and vascular cell adhesion molecule -1.

Whistfatin has also been shown to improve the production of reactive oxygen species (ROS) through the adenine-dinucleotide-dependent phosphate-oxidase pathway, which accelerates vascular disease by causing endothelial dysfunction. Accumulated levels of visfatin in patients with CKD may directly affect the vascular endothelium and cause endothelial dysfunction. In addition, the biomarker improves vascular proliferation and maturation of smooth muscle cells. From all that has been discussed so far, it can be argued that it can be considered as a surrogate marker of endothelial dysfunction in patients with CKD. This thesis is supported by Axelsson et al, who observed a positive association between Visfatin and the endothelial cell adhesion molecule, which is a marker of endothelial dysfunction. [13] The finding was further supported by Yilmaz et al, who observed an improvement in the degree of endothelial dysfunction by assessing the flow-mediated vasodilation of the brachial artery in the first month after kidney transplantation, the improvement being associated with a decrease in blood insulin levels. [178]

In the study by Bessa et al. [154], possible correlations between newly identified adipocytokine - Visfatin and markers of endothelial dysfunction (ED), as well as inflammation in Egyptian patients with CKD are discussed. Serum levels of Visfatin have been shown to be

significantly higher in patients with chronic kidney disease than in the control group of healthy patients, and correlates positively with serum ICAM-1 and VCAM-1 in patients with CKD. These results are in agreement with the findings of Axelsson et al. [13], and Malyszko et al. [104]. They show the relationship between circulating levels of visfatin and endothelial adhesion molecules, discussed as surrogate markers of endothelial dysfunction closely related to the presence of CVD in patients with CKD, as well as an important predictor for them [155]. This observation may be due to the fact that malfunctioning adipose tissue signaling is reflected in an increase in visfatin levels in CKD and may directly affect the vascular endothelium, causing its dysfunction. Kim et al. [83] in 2008 support this link.

In the study by Bessa et al. [154] Stream-mediated vasodilation (FMD) of the brachial artery was used as a method to assess endothelial function. Visfatin is strongly and independently associated with FMD in a multiple regression model. It has been reported to correlate with functional changes in flow - mediated brachial artery vasodilation in patients with CKD [178] and early diabetic nephropathy [176]. Their results are supported by Yilmaz et al. [177], who documented that endothelial function improved in the first month after kidney transplantation, with the degree of improvement associated with a reduction in circulating Visfatin. In view of these results, it is recommended that visfatin be considered as one of the most promising markers of ED in patients with CKD. As FMD is known to depend on the presence of nitric oxide (NO) [70], it is possible that it may be affected by the negative effect of Visfatin on nitric oxide. Impaired endothelium-independent vasodilation (NMD) observed in the study can be explained by the reduction of bioavailable NO to vascular smooth muscle cells and / or smooth muscle dysfunction. This change could also be caused by increased oxidative stress in the vessel wall [33].

The study also discussed the presence of a negative correlation between serum Visfatin and glomerular filtration rate (GFR) in patients with CKD. This suggests that Visfatin levels are affected by renal function. [13] It is discussed that the increased level of visfatin is due to a decrease in GFR. It is not yet known whether its elevated levels may in turn contribute to the progression of renal impairment. The pathophysiological role of visfatin in the kidneys deserves further study.

The paper also discusses that serum Visfatin is positively correlated with inflammatory markers, IL-6 and CRP, but negatively correlated with serum albumin levels in patients with CKD. This confirms the close association of Visfatin with the inflammatory process and hypoalbuminemia, which has also been described in Axelsson et al and Malyszko et al [13,

105]. In the study by Kato et al. [75] also accept these suggestions in hemodialysis patients. This demonstrates that Visfatin can affect both inflammatory status and atherosclerotic changes in this group of patients. There is no doubt that adipokin-managing inflammation plays a key role in CKD. Similar correlations have been reported by Oki et al. [123] in Japanese Americans. It is interesting to note that Visfatin has pro-inflammatory properties. It activates human leukocytes and induces the production of proinflammatory cytokines, including IL-1β, tumor necrosis factor-alpha (TNF-α) and IL-6 [112]. In addition, visfatin expression has been found in synovial fibroblasts in patients with rheumatoid arthritis [24] and in foam cell destabilization over-regulated by foam cell macrophages in atherosclerotic lesions of carotid and coronary vessels [37]. In addition, it may play a significant role in the pathogenesis of diabetic nephropathy [74].

Our results support the claim that low serum albumin levels correlate moderately with CKD progression and in direct proportion to low Visfatin levels.

In the scientific work of Lotfy et al. [98], looked for an association between serum Visfatin and some clinical and biochemical parameters in patients with CKD - body mass index (BMI), blood sugar level, postprandial blood sugar level, hemoglobin level, lipid profile, serum urea, serum creatinine, potassium and phosphorus.

In the patients studied, the body mass index (BMI) is within normal limits, and those with obesity and high BMI affect serum visfatin [142]. A positive association between high BMI and CKD risk has been reported [51, 66]. In addition, high BMI is associated with glomerular hyperperfusion and hyperfiltration, which in turn would lead to renal impairment with proteinuria in obesity-related glomerulopathy. [73, 166]. BMI is considered to be a common, potent and potentially modifiable independent risk factor for CKD [51]. Obesity is also a risk factor leading to progressive loss of renal function in patients with known renal disease [108].От получените резултати се установява съществена разлика в стойностите на ИТМ според отделните нива на Висфатин между двете групи. Регистрират се по-ниски стойности на ИТМ при пациентите, провеждащи заместително лечение на бъбречната функция и характеризиращи се с по-ниски стойности на Висфатин.

In the study by Lotfy et al. [98] reported a significant increase in serum visfatin levels in patients with CKD on hemodialysis compared with controls. The thesis of an increase in its values in patients with CKD has also been confirmed by Axelsson [13] et al, as well as by other studies. Alexon also found a link between Visfatin and soluble vascular adhesion molecule - 1, which is a biomarker for endothelial damage in CKD. In addition, proteinuria, in turn, is an

important predictor of endothelial dysfunction in diabetic nephropathy, with an interesting association between it and Visfatin levels [178].

The published results of Lotfy et al. [98] however, disagree with those of Nüsken et al. [118]. They found low levels of serum visfatin among a cohort of patients with end-stage renal disease treated with hemodialysis, which may be due to the fact that their patients showed a decrease in body fat mass with increased insulin levels. This increase in insulin levels in a study by Neusken and colleagues may explain the strong negative correlation between Visfatin and blood sugar (both fasting and postprandial) in the group of patients in the study by Wahab et al. Further studies of this correlation are needed to investigate the relationship between Visfatin and glycemic status in more detail, as insulin is known to suppress Visfatin levels [4]. Another explanation for low blood sugar may be the glucose-lowering effect of visfatin, as suggested by Naz et al. [116]. On the other hand, there may be other factors that affect the level of visfatin - such as patients' appetite, as well as hydration, which was studied by Chumlea et al. [30]. Dialysis duration and adequacy may also affect visfatin levels in hemodialysis patients due to decreased Visfatin clearance [103].

Our results support Nuksen's thesis, as the recorded values of Visfatin are lower among patients receiving replacement therapy for renal function compared to the predialysis group of patients.

In the study by Lotfy et al, no significant correlation was found between serum visfatin and serum creatinine, as well as a significant negative correlation with blood urea. [98] This is inconsistent with the results obtained by Fathima el al. [47]. In view of this, the study by Wahab et al. the question can be raised: is Visfatin increased due to decreased clearance of Visfatin or due to the ongoing inflammatory process, as suggested by Malyszko et al. Results to date may be partially consistent with this, as there is a lack of correlation between serum visfatin and creatinine and an inverse correlation with urea, despite high serum creatinine and urea in uremic patients. This hypothesis is also supported by the normalization of endothelial dysfunction after kidney transplantation, accompanied by a reduction in circulating visfatin [177].

Lotfy et al. demonstrate a positive association between serum visfatin and serum uric acid, given that it is considered a proinflammatory marker [98]. Ruggiero et al. [139] also confirm the link between uric acid and Visfatin as an inflammatory marker that plays a role in inflammation and atherosclerosis in dialysis patients and answers the question posed in 2013 by Lobo et al: Is there a link between uric acid and inflammation in hemodialysis patients? The report by Lobo et al. agrees with previous results showing that uric acid levels correlate with

inflammatory markers and adhesion molecules in patients undergoing renal replacement therapy [96].

On the other hand, our results show that low uric acid levels are associated with high levels of Visfatin ($r = -0.323$; $p = 0.039$).

Anemia, which develops at the beginning of CKD and is almost constant in patients with CKD stage 5 [78]; In the results of Wahab et al. a significant negative correlation was found between it and serum visfatin values. This association led to the hypothesis that visfatin may have an effect on the pathogenesis of anemic syndrome in CKD. The association that Orasan et al. [124] discussed that the use of l-carnitine in patients with CKD on hemodialysis may lead to a reduction in visfatin and improvement in endothelial dysfunction, but did not cause an improvement in hemoglobin levels or has a causal relationship with anemia, as suggested by Kaygusuz et al. [76]. They believe that high levels of Visfatin may interact with serum iron parameters. Another explanation may be that serum visfatin may play a role in erythropoietin insensitivity in addition to reduced erythropoietin production in patients with renal insufficiency [169].

In the present dissertation as markers related to the anemic syndrome were considered - sEPOR, Vitamin B12, folic acid and serum iron. The results showed a significant difference in folic acid values in the two groups, while the levels of Vitamin B12 did not differ significantly despite the lower values observed in patients in the dialysis group. Significant differences were found in folic acid and Visfatin levels ($p < 0.05$). It is impressive that folic acid levels are higher in patients in the dialysis group, which is a result of the therapy. The analysis of the change in vitamin B12 levels according to Visfatin levels in the two study groups showed that patients in the predialysis group showed a decreasing trend in Vitamin B12 levels with increasing Visfatin levels ($p < 0.05$). On the other hand, with the progression of CKD to the terminal stage, approximately the same levels of Vitamin B12 are maintained, regardless of the levels of Visfatin, which is explained by the implementation of substitution therapy. A significant difference in sEPOR was found between the two groups of patients, as it has higher values in patients in the predialysis group. Considering the relationship between sEPOR and Visfatin, it is shown that there is a moderate positive correlation between the two indicators ($r = 0.336$; $p = 0.035$), which shows that high levels of sEPOR are also associated with high levels of Visfatin in patients in the predialysis group.

The obtained results show that there is a difference in the mean values of sEPOR according to the values of Visfatin, as in the predialysis group the already proven positive

association is presented, while in the dialysis group there is a peak of sEPOR at Visfatin levels between 5.62-15.66 ng / ml (171.38), after which it began to decrease significantly. These results demonstrate the association of sEPOR with the occurrence of anemic syndrome in patients with advanced CKD.

There was a significant difference in the values of serum iron in relation to the threshold value of Visfatin ($p = 0.046$), as at the values of Visfatin below 16.92 ng / ml the levels of serum iron are reduced (respectively 9.06 ± 4.04 to 11.11 ± 6.07), as the trend is preserved in the two study groups.

Regarding the correlation between serum visfatin and lipid profile, Wahab et al, as well as Fathima el al. [47], found a positive correlation between serum visfatin and serum triglyceride (TG) values in patients with CKD, in line with the results of Mu et al. [113]. Mu also showed a positive correlation between visfatin and serum LDL and a negative correlation with serum HDL, as found in the Wahab results. This reaffirms the key role of visfatin in uremia-related atherosclerosis.

A study by Carrero el al [27] in patients with advanced CKD reported that increased circulating visfatin was associated with loss of appetite and decreased circulation in amino acid and triacylglycerol levels. Visfatin has also been shown to be associated with loss of appetite in patients. Anorexia is common in patients with CKD, correlating with loss of appetite. In their study, the authors report that high circulating levels of visfatin are associated with decreased appetite. Visfatin is thought to be involved in the regulation of eating habits. Carrero el al [27] studied visfatin levels among 246 patients with stage 5 CKD starting hemodialysis. The results did not show an association between serum visfatin levels and body mass index (BMI), or serum leptin values. In the group of patients with impaired appetite, visfatin levels are significantly high. They reported a directly proportional correlation between visfatin and the presence of anorexia in this group of patients and low levels of serum albumin, cholesterol and triglycerides, as well as lower essential and non-essential serum amino acids.

However, it is known that the subjective assessment of quality of life is multifactorial and therefore the progression of renal dysfunction may not be the only determining factor for its deterioration. In our study, more socio-demographic factors (age, ethnicity, gender, occupation, education, income) were associated with reduced QOL than physical factors. In addition, it is possible that subjective factors such as adaptation to disease and treatment, satisfaction with medical staff and social support, among others, intervened directly in the assessment of QOL, but were not assessed in this study. The influence of these different factors

on the assessment of QOL may explain the difficulty in establishing a linear relationship with GFR.

The results of the present study show that patients with low levels of Visfatin have poorer heart rate, poor health, more frequent pain that affects their ability to work, have light physical activity, were ill often before starting dialysis treatment, they are often hospitalized and have satisfactory self-esteem.

In conclusion, it can be said that low levels of Visfatin in the current sample are associated with the presence of an inflammatory process, progression of CKD, poor nutritional status, as a result of which patients are in poor health and poor quality of life.

CONCLUSIONS

1) The diagnostic value of Visfatin as a non-invasive marker of inflammation in patients undergoing dialysis treatment has been established.
2) Visfatin levels are significantly reduced in the presence of an inflammatory process in patients receiving dialysis treatment.
3) Although a significant difference in BMI was found between the two study groups, no correlation was found between Visfatin and the nutritional status of the studied patients.
4) Visfatin levels correlate negatively with the duration of dialysis treatment.
5) Low levels of Visfatin are associated with poorer quality of life and poor health.

CONCLUSION

From the analyzes performed and the results obtained, it is now clear that serum visfatin could be a new biomarker for endothelial dysfunction (ED) used in patients with CKD. Possible links between elevated visfatin and inflammation in patients with CKD have been identified.

The results of the present study contradict those of most authors regarding the relationship between Visfatin levels and the inflammatory process, with lower levels of Visfatin in patients with advanced CKD. Lower levels of Visfatin have been shown to be due to the chronic inflammatory process and concomitant changes associated with CKD.

Therefore, with progression to CKD, visfatin levels among these patients most often predict increased mortality. Visfatin can be considered as a new marker in patients with CKD in order to predict complications and quality of life.

CONTRIBUTIONS

Contributions of theoretical character

1) The present study proves for the first time in Bulgaria the diagnostic and prognostic value of Visfatin as a new non-invasive biomarker in patients with different stages of CKD.
2) A detailed review of the literature on the nature of Visfatin and its importance as a non-invasive marker of the inflammatory process.
3) For the first time, a similar monitoring was conducted in the country to determine the level of Visfatin, as well as to determine its relationship with other biomarkers.

Practical contributions

1) A non-invasive biomarker was studied, which is not routinely studied among the patients monitored in the Clinic of Nephrology of the University Hospital "St. Marina "- Visfatin and its role in the diagnosis of the inflammatory process.
2) An algorithm for disease progression and quality of life assessment in patients with CKD undergoing dialysis treatment has been developed and proposed, based on the use of modern non-invasive biomarkers.

PUBLICATIONS RELATED TO THE DISSERTATION

1. Staykova Sv., Petrov P., Grudeva L. Quality of life and regular diet in patients with chronic kidney disease. Scripta Scientifica Medica, 2019;51(3)12-18
2. Petrov A., Benkova-Petrova M., Staykova Sv., Petrov P., Nenova D., Dimieva-Dineva J., Koleva T., Zhelyazkov K., Damyanova D., Ivanova B., Ahmed E., Koleva R. Prevalence of chronic kidney disease among the population of Varna, Valchi Dol municipality and Avren municipality, Current Nephrology, no. 1, Volume 13, 2019, 16-19 (in Bulgarian)
3. Staykova St., Petrov P. Biomarkers and quality of life in patients with chronic kidney disease. Current Nephrology, no. 1, Volume 14, 2020, 17-24 (in Bulgarian)