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Effects of GDF-15 Level in Patients with Membranous Nephropathy*

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ABSTRACT

Objective: Nephrotic syndrome is the most frequent cause of membranous nephropathy (MN) in adults. Growth differentiation factor (GDF)-15 is a cytokine released under stress and associated with increased incidence of chronic kidney disease and/or decreased renal function in conditions such as diabetic nephropathy, IgA nephropathy, lupus nephritis, and primary MN. The diagnosis of MN is made by biopsy, which is an invasive procedure. A non-invasive biomarker is needed for timely and risk-free diagnosis. This study aimed to estimate the GDF-15 level in patients with MN to determine if it can be used as a noninvasive biomarker for the diagnosis of MN.

Materials and Methods: The study included 88 patients with MN. Sera were obtained from peripheral blood collected from the patients. GDF-15 levels were analyzed using enzyme-linked immunosorbent assay (ELISA).

Results: GDF-15 level was high in older patients that in younger patients. The glomerular filtration rate was low in patients with increased GDF-15 levels. Furthermore, a decrease in GDF-15 levels was observed in patients in remission.

Conclusion: GDF-15 level may be used as a biomarker to predict the progression of MN rather than a diagnostic biomarker.

Keywords: Membranous nephropathy, GDF-15, ELISA

INTRODUCTION

Membranous nephropathy (MN), a glomerulopathy, is one of the most important causes of idiopathic nephrotic syndrome in the adult age group (1). A definitive diagnosis can be made with a kidney biopsy. Considering that biopsy protocols differ among countries, there is a regional variance in the incidence of the disease. The disease is seen twice as often in men than in women. MN is a long-term disease that undergoes spontaneous remission in approximately 30% of the patients. Nonetheless, progression to end stage kidney disease (ESKD) may develop within 5 to15 years in 30–40% of the patients with MN (2). Approximately 75% of the MN cases are primary MN (idiopathic-IMN) and 25% are secondary MN (due to autoimmune diseases, infections, or malignancies).

Growth differentiation factor (GDF)-15 is a cytokine produced in several tissues in response to stress and whose levels increase in response to stimuli in some diseases. However, the underlying mechanisms remain unknown. GDF-15 levels are significantly increased in patients with

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IMN and negatively correlated with the glomerular filtration rate (eGFR) (3, 4).

Increased level of circulating GDF-15 has been linked to a decrease in estimated glomerular filtration rate (eGFR), kidney damage, and progression to ESKD, and it may be a marker of chronic kidney damage (5, 6). Based on the studies conducted to date, we hypothesized that high circulating GDF-15 levels may be used as a diagnostic biomarker of MN in patients.

MATERIALS AND METHODS

Study Population

Eighty-eight adult patients, who presented to the Department of Nephrology, Istanbul Faculty of Medicine, Istanbul University (Istanbul, Turkiye) between 2010 and 2017 and were diagnosed with MN based on biopsy examination, were included in the study. The study was approved by the clinical experiments local ethical committee of Istanbul University Istanbul Faculty of Medicine (No: 2018/1260; date: 03/10/2018), and informed written consent was obtained from the study participants. The study was conducted in accordance with the principles of the 1975's Helsinki Declaration. Blood samples were obtained at the time of diagnosis, and 51% of patients had stage II of MN. No control group was included in the study. The reference range of GDF-15 was 399–1335 pg/mL based on the comparison of sex, lifestyle, and biological factors between healthy individuals (7, 8).

Table 1: Demographic data of the patients

Characteristics	
Age, year	53.59 ± 13.82
Age at the time of diagnosis	46.3 ± 13.5
Sex, Male / Female, n	51/37
BMI, kg/m^2	78.90 ± 14.7
Systolic blood pressure, mmHg	131.42 ± 19.2
Diastolic blood pressure, mmHg	81.13 ± 12.5
Serum albumin, g/dL	2.80 ± 0.8
Serum creatinine, mg/dL	0.9 ± 0.4
Serum creatinine on follow-up, mg/dL	1.2 ± 1.1
Hemoglobin, g/dL	12.93 ± 2.3
eGFR, mL/minute/1.73m^2	102.21 ± 52.1
Proteinuria, mg	5677.79 ± 3545.6
Proteinuria on follow-up, mg	2869.82 ± 3003.5

Data are presented as mean ± SD unless stated otherwise
BMI, body mass index; eGFR, estimated glomerular filtration rate

Clinical Parameters

Proteinuria, proteinuria at follow-up, serum albumin, serum creatinine, serum creatinine at follow-up, eGFR hemoglobin, serum albumin, systolic blood pressure, diastolic blood pressure, BMI (kg/m^2), remission and ESKD were compared with GDF-15 level. Immunosuppressive agents such as mycophenolate mofetil, cyclophosphamide, cyclosporine, and steroids and antihypertensive drugs such as angiotensin receptor blockers (ARBs) and/or angiotensin-converting enzyme inhibitors (ACEIs) were administered to the patients during the treatment process.

Measurement of Serum GDF-15 Levels

Blood was obtained once from the patients at the diagnosis. The serums obtained from the blood samples were stored at -20°C until they were analyzed. The GDF-15 levels were measured using enzyme-linked immunosorbent assay (ELISA) (Human GDF-15; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacture's instructions, and the results were read at 450 nm.

Statistical Analyses

The sample size was calculated using an online-based software (http://clincalc.com/stats/SampleSize.aspx). Based on the mean GDF-15 levels in literature, at least 81 participants were required for a 20% difference (significant difference), an alpha error of 0.05, and 80% power. However, we included 90 patients to compensate for patients who may be lost to

Table 2: Relationship between serum GDF-15 levels and the demographic and clinical parameters of the patient

Clinical parameters	**N**	**r**	**p-value**
Age, year	88	0.229	***0.032***
Proteinuria, mg	80	0.076	0.501
Proteinuria on follow-up, mg	74	0.333	***0.004***
Serum creatinine, mg/dL	88	0.268	***0.012***
Serum creatinine on follow-up, mg/dL	82	0.209	0.059
eGFR, mL/minute/1.73m^2	88	–0.249	***0.019***
Hemoglobin, g/dL	86	–0.06	0.580
Serum albumin, g/dL	88	–0.11	0.308
Systolic blood pressure, mmHg	53	0.79	0.575
Diastolic blood pressure, mmHg	53	–0.27	0.850
BMI, kg/m^2	52	0.052	0.713

eGFR, estimated glomerular filtration rate; BMI, body mass index; N, number; r, correlation coefficient, p<0.05 is significant.

follow-up. Statistical analyses of the data were performed using SPSS (version 29.0.0.0; IBM, Armonk, New York, USA). The Kolmogorov Smirnov test was used to assess normality of distribution of the continuous data. The normally distributed data are presented as mean ± standard deviation, and the non-normally distributed data are presented as median (minimum–maximum). To determine the relationship between a dependent variable and one or more explanatory variables, linear regression analysis was performed. The Pearson correlation test was used to assess the relationship between age and GDF-15 levels. The Chi-square and Fisher's exact tests were used to evaluate categorical data, which are presented as numbers and percentages values, between the patient groups. A p-value of <0.05 was considered statistically significant. A receiver operating characteristic (ROC) curve demonstrating the prognostic sensitivity and specificity of GDF-15 levels was constructed using the eGFR value, which is an indicator of kidney disease progression (>90 mL/min and <90 mL/min). A GDF-15 value of >6500 pg/mL demonstrated 81% specificity and 77.8% sensitivity in predicting disease progression. The patients were segmented into two groups depending on these cut-off values. Group 1 included patients with a GDF level of <6500 pg/mL, and Group 2 included patients with a GDF level of >6500 pg/mL. A GDF-15 cut-off value of 6500 pg/mL was specified to predict disease progression. The AUC value (0.684) for the GDF-15 level was within the confidence interval (95% CI) of 0.529 to 0.838 (p=0.003) (Figure 1).

RESULTS

The mean age of the patients was 53.59 ± 13.82 years (range: 25–77), and the mean age of the patients at time of diagnosis was 46.30 ± 13.53 years (range: 19–70). The patient group included of 51 males (58%) and 37 females (42%) (Table 1).

Demographic and Clinical Parameters and the GDF-15 Levels

The mean follow-up period in the patient group was 87.75 ± 29.30 months (range: 48–180; Inter Quartil Range: 48). There was no significance between the serum GDF-15 level and

Table 3: Comparison of patient characteristics in Groups 1 and 2

		Group 1 (n = 53)	Group 2 (n = 35)	p-value
Age, year		50.83 ± 13.6	57.77 ± 13.3	**0.002***
Sex, Male / Female, n		30/23	21/14	0.752
Age at the time of diagnosis		43.26 ± 13.4	50.89 ± 12.6	**0.009***
Weight, kg		79.2 ± 15.3	78.4 ± 14	0.847
Systolic blood pressure, mmHg		131.4 ± 21	131.5 ± 16.3	0.519
Diastolic blood pressure, mmHg		82.7 ± 12.8	79.7 ± 12.2	0.426
Serum albumin, g/dL		2.78 ± 0.9	2.84 ± 0.8	0.745
Serum creatinine, mg/dL		0.75 ± 0.3	1.1 ± 0.5	**<0.0001**
Serum creatinine on follow-up, mg/dL		0.85 ± 0.31	1.7 ± 1.67	**0.001***
Hemoglobin, g/dL		13.3 ± 2.6	12.4 ± 1.6	0.066
eGFR, mL/minute/1.73 m^2		116.1 ± 51.01	81.2 ± 46.5	**0.002***
Proteinuria, mg		5474.8 ± 3423.5	5998.7 ± 3765.4	0.523
Proteinuria on follow-up, mg		2014.9 ± 2104.2	4196.4 ± 3683	**0.002***
Remission	%	39/11 73.58/20.75	16/17 37.14/48.57	**0.005***
ESKD	%	50/1 94.33/1.88	30/3 85.71/8.57	0.134
ACEI or ARB	%	2/48 3.7/90.56	4/29 11.42/82.85	0.162

All data are presented as mean ± SD unless otherwise stated

eGFR, estimated glomerular filtration rate; ESKD, end stage kidney disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; SD, standard deviation.

The Student's t-test, Chi-square and Fisher's exact test were used. *: p<0.05.

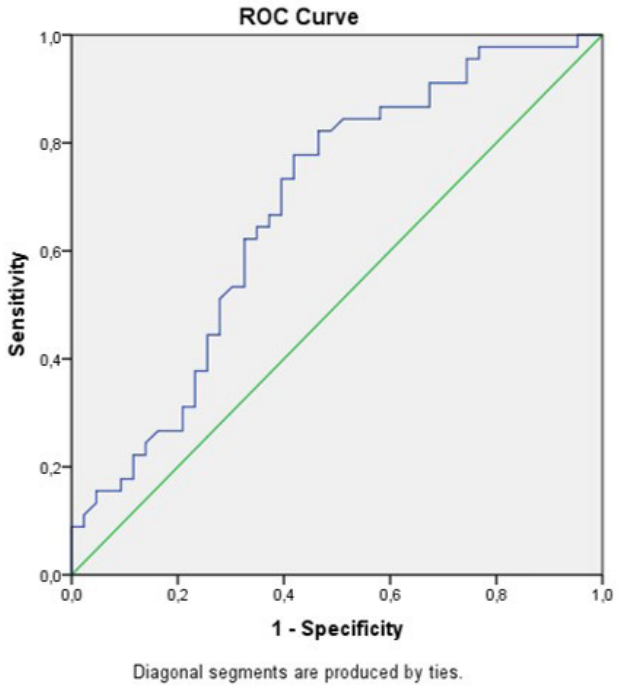

Figure 1. The ROC for GDF-15 level in patients with MN. The AUC value: 0.684; 95CI: 0.529-0.838; p-value: 0.003

proteinuria, follow-up creatinine level, albumin level during biopsy, hemoglobin level, and systolic and diastolic blood pressures (p>0.05). There was a positive correlation between increasing serum GDF-15 levels and increasing age (p=0.032), serum creatinine level (p=0.012) and follow-up proteinuria (p=0.004). However, there was a negative correlation between increasing serum GDF-15 levels and eGFR (p=0.019) (Table 2).

Association Between GDF-15 Levels and Progression of Renal Disease

The patients in Group 2 were older (p=0.009), had higher serum creatinine levels (p<0.0001), higher follow-up proteinuria (p=0.006), and lower eGFR (p=0.002) than the patients in Group 1 (Table 3).

Logistic regression analysis of the GDF-15 level and age revealed a significant positive relation (p=0.024; OR, 1.040; 95% CI, 1.005–1.076). The Pearson correlation test demonstrated a low correlation between GDF-15 levels and age (r=0.247, p=0.020).

Association Between GDF-15 Level and Prognosis

The GDF-15 level was statistically significantly low in patients in remission (p=0.002). However, it was higher in patients with an eGFR of <90 mL/min (p=0.003). There was no significant correlation between the GDF-15 level and development of ESKD (p=0.392) and treatment (ACEI/ARB, p=0.36) (Table 4). Approximately 62.5% (n = 55) of the patients were administered immunosuppressive therapy.

DISCUSSION

MN is the most common primary glomerular disease worldwide. Although spontaneous remission may occur, ESKD may develop in 30–40% of patients with MN within 5–15 years (2). Although MN is typically a disease of adults, rarely, it can be seen in children. The incidence is two fold more in adult men than in women. The progression of MN is faster in male and older adults than in women and younger adult patients (9). In our study, there was no significant difference in gender in the included patients. Furthermore, we did not detect a

Table 4: Relationship of GDF-15 level with poor prognosis.

			GDF-15 levels (Min–Max)	Median	p-value
eGFR	90* ≤	n = 43	1287.58–26760.73	7285.76	**0.003***
	90* >	n = 45	744.36–19332.63	4500.22	
Remission	(+)	n = 55	936.74–26760.73	4357.37	**0.002***
	(–)	n = 28	2300.22–25998.88	7369.09	
ESKD	(–)	n = 80	936.74–26760.73	5631.10	0.392
	(+)	n = 8	744.36–10118.92	7785.73	
ACEI or ARB	(–)	n = 6	1096.21–12118.79	7266.01	0.360
	(+)	n = 77	936.74–26760.73	6313.29	

GDF, growth differentiation factor; eGFR, estimated glomerular filtration rate; ESKD, end stage kidney disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker

Mann–Whitney U Test was used to perform statistical analysis. *: p<0.05.

relationship between disease progression and sex or age. This may be attributed to the difference in disease progression among patients and the limited number of patients with advanced disease stage.

Under normal circumstances, GDF-15 is expressed in low concentrations in most organs. However, GDF-15 is expressed in high concentrations following injury to organs such as the heart, liver, and kidney. Recently, GDF-15 expression has been found to be increased in damaged organ tissues such as the heart and kidney in addition to other inflammatory markers (3, 10, 11). In a study which included patients with type 1 diabetes mellitus, there was no significant difference in the plasma concentrations of GDF-15 between the male and female patients (12). However, GDF-15 is positively correlated with age (5, 13-15). Tuegel et al. demonstrated that female gender, advanced age, and smoking were associated with elevated GDF-15 levels as independent factors (16). In this study, the GDF-15 level was positively correlated with age, which is consistent with the findings in literature. However, there was no significant difference between the serum GDF-15 level and the gender of the patients.

Ham et al. demonstrated that serum hemoglobin and eGFR values were low, and age and serum creatinine values were high in patients with a serum GDF-15 level of >2.15 ng/mL. Furthermore, they demonstrated that GDF-15 levels are negatively correlated with kidney function and positively correlated with disease progression in patients with IMN (3). Other studies have also demonstrated elevated GDF-15 levels in patients with chronic kidney injury and/or disease progression to ESKD (5, 6, 17). Wu et al. demonstrated that GDF-15 levels are significantly higher in patients with IMN than in healthy individuals and that the GDF-15 level is associated with kidney functions. Although GDF-15 level alone is not a prognostic biomarker, its concentrations may be utilized as a biomarker of the degree of renal failure in patients with IMN patients (4).

In our study, ESKD developed in eight of the 88 patients. Furthermore, we found that the serum GDF-15 value was >6500 pg/mL in 75% of the patients who developed ESKD. However, this difference was not statistically significant, and this may be attributed to the low number of patients with ESKD included in the study. Follow-up creatinine levels and proteinuria were high in patients with GDF-15 levels >6500 pg/mL than in patients with GDF-15 levels <6500 pg/mL. This indicated that there is a relationship between GDF-15 concentration and disease progression. Determining serum levels of GDF-15 is useful for risk stratification, especially in patients with normal creatinine levels (12, 18). Studies have indicated that plasma GDF-15 levels is positively correlated with serum creatinine level as well as proteinuria (9, 19). Similarly, in our study, we found a positive correlation between GDF-15 levels and follow-up levels of proteinuria and serum creatinine and an inverse correlation between GDF-15 level and eGFR. Further, there was a significant relationship between serum GDF-15 concentration at the time of diagnosis and remission; patients with low GDF-15 concentrations had higher chances of remission.

The GDF-15 levels are reportedly significantly higher in patients with anemia (World Health Organization definition, male hemoglobin level <13 mg/dL and female hemoglobin level <12 mg/dL) than in patients without anemic (18-20). In our study, hemoglobin levels were low and GDF-15 concentrations were high in patients with an eGFR >90, which is consistent with the findings in literature.

In conclusion, herein, we aimed to determine whether GDF-15 level can be used as a biomarker to diagnose MN in patients without the need for biopsy. Our study findings revealed a positive correlation between GDF-15 level and follow-up proteinuria and a negative correlation between GDF-15 and eGFR and remission. These findings indicate that GDF-15 concentration can be used as an indicator of the course of the disease rather than as a diagnostic biomarker.

Ethical Committee Approval: This study was approved by the clinical experiments local ethical committee of Istanbul University Istanbul Faculty of Medicine (No: 2018/1260; date: 03/10/2018).

Informed Consent: Informed written consent was obtained from the study participants.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.A., H.Y., C.K.C.; Data Acquisition- A.A., E.D., Data Analysis/Interpretation- A.A., S.U.A., H.S.C., C.K.C., Drafting Manuscript-AA, HY, CKC, Critical Revision of Manuscript- F.S.O., HY, C.K.C., Final Approval and Accountability- H.Y., C.K.C., Technical or Material Support- E.D., H.Y., C.K.C., Supervision- H.Y., H.S.C., C.K.C.

Conflict of Interest: The authors declare that they have no conflict of interest.

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