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Original Article

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Relationship of Serum Pro-Gastrin-Releasing Peptide with Kidney Fibrosis and Proteinuria

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ABSTRACT

Background: Interstitial fibrosis/tubular atrophy (IFTA) plays a crucial role in the pathogenesis of chronic kidney disease. Pro-gastrin-releasing peptide (proGRP), a neuropeptide, plays a role in stimulating fibroblast activity. This study explores the association between serum proGRP levels and IFTA severity.
Methods: The patient group consisted of 49 patients (23 males; mean age 44.1 ± 15 years) who underwent kidney biopsy and the control group comprised 20 healthy volunteers (8 males; mean age 43.4 ± 7.2 years). Pro-gastrin-releasing peptide was measured using a commercially available Elabscience Human Pro-Gastrin-Releasing Peptide enzyme-linked immunosorbent assay kit. The scoring system was implemented based on the percentage of IFTA severity in kidney tissue samples.
Results: Pro-gastrin-releasing peptide levels were observed to be significantly elevated in the patient group compared to the controls [30.0 (22.1-48.1) U/L vs 19.7 (15.1-30.3) U/L, (P = .004)]. A statistically significant positive correlation was identified between proGRP level and the presence of proteinuria (P = .012).
Conclusion: The study demonstrated that proGRP levels might increase in the presence of kidney fibrosis and proteinuria.
Keywords: Kidney fibrosis, proteinuria, serum pro-gastrin-releasing peptide

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INTRODUCTION

Proteinuria and interstitial fibrosis/tubular atrophy (IFTA) are key contributors to the progression of chronic kidney disease (CKD). Interstitial fibrosis/tubular atrophy is characterized by increased kidney fibroblast numbers and excessive aggregation of activated extracellular matrix components.[1]

Tubular cells respond to severe or persistent proteinuria with the production of cytokines, which leads to increased fibroblast activity and migration of macrophages.[2] Furthermore, proteinuria induces apoptosis in tubular cells,[3] as well as a change towards a mesenchymal phenotype called epithelial-mesenchymal transition. This results in increased extracellular matrix and fibrosis.[4]

A neuropeptide called gastrin-releasing peptide (GRP) is released by gastrin in the stomach.[5] It primarily binds to GRP receptor (GRPR), which influences gut function through controlling the release of enteric neurotransmitters.[5] Pro-gastrin-releasing peptide is a neuropeptide involved in various physiological and pathophysiological processes in the body.[6] Gastrin-releasing peptide binds with the GRPR to induce intracellular changes that influence multiple cell behaviors and biological processes, including neuroendocrine regulation,[7,8] gastrointestinal system functions, cancer growth,[9] cell proliferation, and immune cell regulation.[10,11] Notably, GRP/GRPR may serve as a potential therapeutic target in inflammatory diseases.[12] Recent studies have demonstrated that serum proGRP levels in patients with CKD are higher compared to individuals with normal kidney function.[13]

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Monitoring of tubulointerstitial damage markers in patients with glomerulopathy may help both early detection in the onset of CKD and early regulation of nephroprotective therapy, which may lead to favorable outcomes in CKD management. According to existing literature, there is no research assessing the connection between kidney fibrosis and serum proGRP in the literature. Thus, it was sought to examine the connection between serum proGRP level, proteinuria, and the presence and severity of IFTA in patients undergoing kidney biopsy.

MATERIAL AND METHODS

This cross-sectional study was conducted following approval from the Ethics Committee of Hitit University Faculty of Medicine (Date: November 29, 2021; Approval No: 504). All patients gave written informed consent. The patient group consisted of 49 patients (23 males; mean age 44.1 ± 15 years) who underwent kidney biopsy and the control group comprised 20 healthy volunteers (8 males; mean age 43.4 ± 7.2 years).

Patients over 18 years of age with unexplained proteinuria and/or hematuria or persistent decreased estimated glomerular filtration rate (eGFR) in addition to these findings, underwent kidney biopsy. Controls were age and gender matched volunteers with normal eGFR. Demographic, somatometric, and laboratory data were recorded.

Patients with a history of acute or chronic active infectious disease, uncontrolled hypertension, thyroid dysfunction, malignancy, acute or chronic organ failure outside of kidney, surgical operation within the last 3 months, autoimmune or inflammatory disease, pregnancy, and kidney transplantation were excluded.

Eight-hour fasting blood and 24-hour urine specimens were collected from all participants 1 day before kidney biopsy. Serum obtained after centrifugation of blood samples at 1310 g for 10 minutes was analyzed for biochemical parameters including blood urea nitrogen (BUN), creatinine, glucose, uric acid, albumin, lipids, and C-reactive protein (CRP) levels. Simultaneously, a serum sample was obtained and stored at −80°C for subsequent measurement of proGRP levels.

MAIN POINTS

- In this initial study assessing the association between kidney fibrosis and serum pro-gastrin-releasing peptide (proGRP), higher serum proGRP levels were found in patients with persistent proteinuria.
- It has been shown that there may be a positive correlation between the severity of kidney fibrosis and serum proGRP.
- In addition to its clear relationship with proteinuria, it is necessary to show whether serum proGRP has an effect on predicting the presence and severity of kidney fibrosis.

Biochemical parameters were analyzed using the Beckman Coulter AU 5800 autoanalyzer, while complete blood count analysis was conducted with the Mindray BC-6800 hematology analyzer. Estimated glomerular filtration rate values were computed using the Chronic Kidney Disease Epidemiology Collaboration equation.[14] Serum CRP and erythrocyte sedimentation rate (ESR) levels were quantified using the nephelometric and Westergren methods, respectively.[15]

Human proGRP concentrations (Cat. No: E-EL-H2059, Elapscience Biotechnology Co. Ltd) were assessed through the use of commercially available enzyme-linked immunosorbent assay kits, following the manufacturers' instructions. The coefficient of variation within the assay for proGRP assays was <10%. The sensitivity for proGRP assays was 9.38 ng/mL. The assay range of proGRP was 15.63-1000 ng/mL.

Kidney Biopsy Procedure and Histopathologic Evaluation

Kidney tissue samples were obtained under ultrasound guidance using semi-automated Tru-cut 16G 15 cm biopsy needles (Ref No: GSN1615, Geotek Medical Ltd. Türkiye). Biopsy samples were preserved in a 10% neutral-buffered formalin, thereafter dried in graded alcohols, and subsequently embedded in paraffin. For diagnostic and histopathological evaluation, 0.4 mm thick sections were prepared from the tissue samples, stained with hematoxylin/eosin, periodic acid-Schiff (PAS), and trichrome, and subsequently examined using immunofluorescence microscopy. To evaluate IFTA, cortical regions in PAS and trichrome-stained sections were examined sequentially under a Nikon microscope at 200× magnification. Histopathologic evaluations were performed by 2 pathologists unaware of the serum proGRP results and disease. The level of interobserver agreement between Aysel Çolak and Esra Şengül was analyzed. In case of different decisions, biopsy specimens were re-evaluated simultaneously by Aysel Çolak and Esra Şengül. Biopsy material containing 10% or more glomeruli was considered adequate. The extent of IFTA was assessed by determining the percentage of areas in the form of tubular dilatation, fibrosis, and interstitial infiltration per area of cortex. The IFTA score was calculated utilizing a scoring system that incorporated the following parameters: IFTA percentage (1 <= 10%; 2 = 10%-24%; 3 = 25%-50%; 4 => 50%). Evaluation of glomerulosclerosis was calculated using a similar scoring system and mean values.[16,17]

Statistical Analysis

Statistical analyses were executed using the licensed SPSS v. 23.0 (IBM SPSS Corp.; Armonk, NY, USA), on the Windows platform . To examine whether the groups followed a normal distribution, Kolmogorov–Smirnov and Shapiro–Wilk analyses were performed. Data are reported as mean ± SD for Gaussian-distributed variables and as median (25th-75th percentiles) for non-Gaussian-distributed variables. Categorical variables were analyzed utilizing the chi-square test and reported as numbers and percentages. One-way ANOVA with Bonferroni correction and independent samples t-test were used for normally

distributed groups, while non-normally distributed groups were compared using Kruskal–Wallis and Mann–Whitney *U* tests. The correlation between the parameters was examined using Spearman correlation analysis. Additionally, the independent effect of each variable in relation to the presence of IFTA was analyzed using univariate logistic regression analysis, followed by the evaluation of statistically significant parameters through multivariate logistic regression analysis. A *P*-value of less than .05 was considered statistically significant for all comparisons.

RESULTS

We have included 49 patients and 20 volunteers with normal kidney function. There were no statistically meaningful differences in the mean age, body mass index, gender distribution, and blood pressure between the groups. Proteinuria and serum proGRP values showed statistically meaningful differences across the groups ($P < .01$, $P = .04$, respectively). The relationship between serum proGRP levels among the groups is presented in Figure 1. The clinical characteristics of the groups are outlined in Table 1.

Patients were stratified into groups based on IFTA scores of 1, 2, 3, and 4. There were 19 (38.7%), 16 (32.6%), 8 (16.3%), and 6 (12.2%) patients in these groups, respectively. Since the number of patients with IFTA scores 3 and 4 was low, these groups were combined and statistically evaluated together. It was observed that eGFR decreased with increasing IFTA score and was statistically significant between groups 1-3 and groups 1-2 ($P = .027$, $P < .001$, respectively). The amount of proteinuria in IFTA scores 3 and 4 was statistically significantly higher than in IFTA score 1 ($P = .016$). Although the serum proGRP levels were elevated in the group with IFTA score 1 compared to the other groups, the difference did not achieve statistical significance. Pro-gastrin-releasing peptide levels were [38.5 (28.3-65.0); 23.2 (19.5-48.6); 27.2 (22.2-36.5)] in the groups with IFTA scores 1, 2, and 3+4, respectively ($P = .209$), (Figure 2)]. A detailed comparison of the

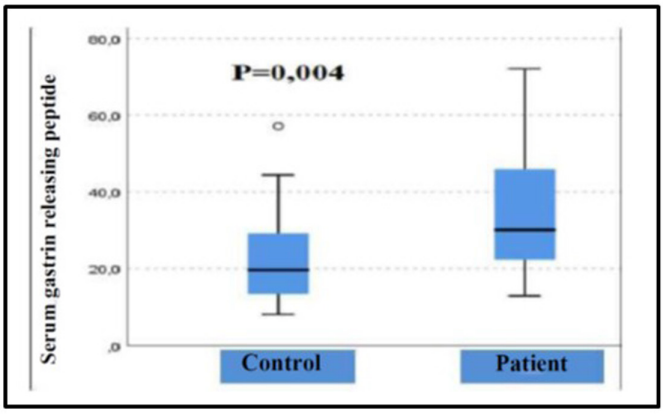

Figure 1. Serum progastrin-releasing peptide levels of the patient and control group.

Table 1. Demographic and Laboratory Characteristics of Patient and Control Groups

Parameters	Patient (n = 49)	Control (n = 20)	*P*
Age (years)	44.1 ± 15.0	43.4 ± 7.2	.815
Gender (male)	23 (46.9)	8 (40)	.599
BMI (kg/m$^{2)}$	28.4 ± 4.6	27.4 ± 3.9	.367
SBP (mm/Hg)	123 (111-135)	123 (109-126)	.908
DBP (mm/Hg)	76 (68-84)	77 (70-84)	.781
DM, n(%)	9 (18.4)	–	
HT, n(%)	17(34.7)	–	
Smoking, n(%)	6 (12.2)	7 (35)	.042
Histopathological diagnoses*			
FSGS, n(%)	11 (22.4)	–	
IgAN, n(%)	8 (16.3)	–	
DNP, n(%)	7 (14.3)	–	
MCD, n(%)	6 (12.2)	–	
MGN, n(%)	4 (8.2)	–	
Other, n(%)	13 (26.4)	–	
RAAS blockers, n(%)	11(22.4)	–	
Hemoglobin (g/dL)	12.6 ± 2.2	14.4 ± 1.43	.002
Glucose (mg/dL)	95 (90-104)	92 (81 -102)	.211
BUN (mg/dL)	21 (14-27)	11 (9-13)	<.001
Creatinine (mg/dL)	1 (0.7-1.6)	0.7 (0.6-0.8)	<.001
eGFR (mL/min/1.73 m^2)	89 (47-106)	108 (102 - 117)	<.001
Sodium (mmol/L)	139.4 ± 2.8	139.1 ± 1.2	.545
Potassium (mmol/L)	4.4 ± 0.4	4.2 ± 0.1	.038
Calcium (mg/dL)	8.8 ± 0.8	9.1 ± 0.3	.022
Phosphorus (mg/dL)	3.7 ± 0.8	3.7 ± 0.2	.789
Albumin (g/L)	3.3 ± 0.9	4.3 ± 0.3	<.001
Uric acid (mg/dL)	6.2 ± 1.4	4.8 ± 1.5	<.001
HDL-c (mg/dL)	50 ± 16	53 ± 12	.468
LDL-c (mg/dL)	134 (97-180)	111 (87-133)	.102
NLO	2.04 (1.55-2.84)	1.57 (1.24-2.26)	.039
ESR (mm/hour)	31 (15-60)	8 (5-17)	<.001
ysCRP (mg/L)	3.4 (2-10)	4 (3-5.5)	.989
Proteinuria (mg/day)	3591 (2000-6000)	62 (50-72)	<.001
ProGRP (U/L)	30.0 (22.1-48.1)	19.6 (12.7-30.3)	.004

Categorical data are presented as frequencies and percentages; continuous variables are presented as mean ± SDs or median and interquartile ranges (IQR: 25-75) depending on their distribution.
BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; DM, diabetes mellitus; DNP, diyabetic nefropathy eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; FSGS, focal segmental glomerulosclerosis; HDL-c, high-density lipoprotein cholesterol; HT, hypertension; IgAN, IgA nephropathy; LDL-c, low-density lipoprotein cholesterol; MCD, minimal change disease; MGN, membranous glomerulonephritis; NLO, neutrophil to lymphocyte ratio; ProGRP, pro-gastrin-releasing peptide; RAAS, renin angiotensin aldosterone system; SBP, systolic blood pressure.

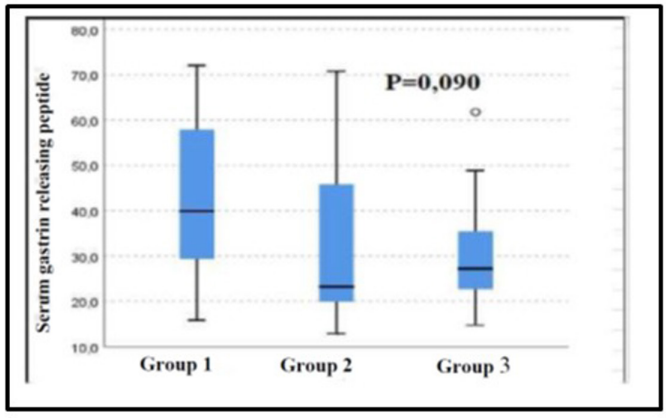

Figure 2. Serum progastrin-releasing peptide levels of groups formed according o the severity of interstitial fibrosis/tubular atrophy.

clinical characteristics among the patients, categorized by IFTA scores, is provided in Table 2.

Serum proGRP levels were found to be positively correlated with proteinuria (r = 0.304, *P* = .012), while the positive correlation did not reach statistical significance with eGFR (r = 0.067, *P* = .099). Detailed correlation analysis of variables with serum proGRP is presented in Table 3.

Interstitial fibrosis/tubular atrophy score was correlated negatively with eGFR and positively with proteinuria, ESR, hsCRP, and phosphorus (Table 4).

Univariate and multivariate logistic regression analyses were used to evaluate the link between the presence of IFTA and serum proGRP levels and proteinuria, and the association was found to be borderline insignificant (Table 5).

To identify the severity of kidney fibrosis by serum proGRP levels, Receiver Operating Characteristic (ROC) curve analysis was employed to identify the ideal cut-off values with the greatest discriminatory power. Serum proGRP levels demonstrated the greatest area under the ROC curve [0.727 (0.593-0.861), *P* = .003, (Figure 3)]. The cut-off point for serum proGRP of 23.3 had a specificity of 65% and sensitivity of 66.9%.

DISCUSSION

Based on the authors' knowledge, this is the first study examining the relationship between IFTA score and serum proGRP levels in patients with persistent proteinuria. Serum proGRP levels of the patients were markedly higher compared with the healthy controls. Serum proGRP and eGFR levels were negatively correlated with IFTA stage, while ESR and CRP levels, markers of inflammation and proteinuria, were positively correlated. It has been demonstrated that serum proGRP levels can independently be linked with the presence of IFTA. This study showed that serum proGRP levels may be elevated in the occurrence of kidney fibrosis and proteinuria.

Proteinuria may cause CKD and progression of CKD by inducing tubulointerstitial damage with a direct toxic effect.[18] Inflammation and fibrosis may occur as a consequence of changes in tubular cell growth, apoptosis, and gene transcription due to proteinuria.[19] It is well established that proteinuria contributes to inflammation and kidney fibrosis. In this study, a statistically significant positive correlation was found between serum proGRP levels and proteinuria. The mechanisms through which increased inflammatory mediators and subsequent renal fibrosis may occur have been explained via the serum proGRP–GRPR axis.[20] Based on this finding, it is suggested that elevated proteinuria and high serum proGRP levels may contribute to the progression of kidney fibrosis.

Various structural changes occur in the CKD process, including extracellular matrix deposition, interstitial fibrosis, tubular atrophy, and tubular degeneration.[21,22] The negative correlation between IFTA severity and eGFR detected in this study is consistent with the previously published literature data.

In a cell culture model, proGRP may contribute to pulmonary fibrosis through its proliferative and fibrotic effects on lung fibroblasts.[23] It has been shown that the external addition of proGRP to cell cultures made from fibroblast-like synoviocytes obtained from the collagen-induced arthritis model in mice causes increased activity in these cells.[24] Studies have shown that proGRP concentrations in individuals with idiopathic pulmonary fibrosis[25] and sarcoidosis[26] are significantly elevated compared to healthy controls. The authors of these studies emphasized the effect of proGRP in the formation of fibrosis through fibroblast activation and stated that proGRP might be considered a potential therapeutic target in these diseases.

Studies have demonstrated that the expression levels of proGRP and GRPR are significantly elevated in patients with hyperuricemic nephropathy (HN) as well as in HN mouse models. It has been shown that GRP/GRPR promotes the development of HN by activating the NF-κB pathway, thereby suppressing ATP-binding cassette subfamily G member 2 (ABCG2)/PDZ domain-containing 1 (PDZK1) levels and enhancing Transforming growth factor beta (TGF-β)/Smad3 signaling. Furthermore, GRPR overexpression has been found to increase the mRNA levels of fibrotic markers such as Collagen-1,Alpha–Smooth Muscle Actin (α-SMA), and TGF-β1, along with inflammatory cytokines including Interleukin-1 beta (IL-1β) and TNF-α. The results showed that hyperuricemia-induced kidney injury, inflammation, and fibrosis are mediated via GRPR-related mechanisms. In vivo, GRPR knockdown, GRPR conditional knockout, or GRPR inhibition effectively attenuated hyperuricemia-induced kidney damage, inflammation, and fibrosis.[20]

Table 2. Comparison of Demographic and Biochemical Data of Patients According to Interstitial Fibrosis/Tubular Atrophy Scores

Parameters	Score 1 (n = 19)	Score 2 (n = 16)	Score 3 + 4 (n = 14)	*P*
Age (years)	42 ± 18	46 ± 16	45 ± 10	.748
Gender (male)	9 (47.4)	6 (37.5)	8 (57.1)	.377
BMI (kg/m^2)	29.2 ± 4.4	27 ± 4.6	28.9 ± 4.8	.327
SBP (mm/Hg)	123 (112-134)	120 (109-131)	128 (113-143)	.212
DBP (mm/Hg)	75 (67-83)	74 (66-82)	78 (69-87)	.428
DM, n(%)	3 (15.8)	3 (18.8)	3 (21.4)	1.000
HT, n(%)	6 (31.6)	5 (31.3)	6 (42.9)	.803
Smoking, n(%)	2 (10.5)	1 (6.3)	3 (21.4)	.553
RAAS blockers, n(%)	4 (21.1)	3 (18.8)	4 (28.6)	.831
Hemoglobin (g/dL)	13.6 ± 1.9[a]	11.8 ± 2.5	12.1 ± 1.7	.044
Glucose (mg/dL)	96 (88-106)	94 (90-100)	98 (88-128)	.571
BUN (mg/dL)	16 (12-22)[b]	18 (12-30)	29 (22-47)	.002
Creatinine (mg/dL)	0.8 (0.6-0.9)[c]	1.1 (0.8-1.4)[d]	1.9 (1.4-3.2)	<.001
eGFR (mL/min/1.73 m^2)	106 (94-120)[e,f]	80 (55-100)	33 (20-61)	<.001
Sodium (mmol/L)	139 ± 2.8	139.5 ± 2.6	139.8 ± 3	.713
Potassium (mmol/L)	4.3 ± 0.4	4.4 ± 0.5	4.3 ± 0.3	.855
Calcium (mg/dL)	9 ± 0,7	8,7 ± 0,9	8,5 ± 0,6	.229
Phosphorus (mg/dL)	3.4 (3-4)	3.55 (3.3-4)	3.7 (3.27-4.45)	.236
Albumin (g/L)	3.5 ± 0.9	3.1 ± 1.1	3.1 ± 0.7	.251
Uric acid (mg/dL)	6.3 ± 1.4	6.1 ± 1.7	6.4 ± 1.2	.870
HDL-c (mg/dL)	45 (40-59)	43 (35-51)	34 (50-68)	.530
LDL-c (mg/dL)	135 (90-197)	131 (90-161)	128 (104-239)	.711
NL rate	1.87 (1.5-2.7)	2.59 (2.0-3.6)	1.74 (1.5-2.9)	.102
ESH (mm/hour)	22 (10-29)[g]	35 (13-63)	47 (33-63)	.009
hsCRP (mg/L)	3.1 (0.7-5)[h]	4 (27-10)	7.5 (3.1-23)	.039
Proteinuria (mg/day)	3352 (1640-4764)[i]	3294 (2000-6000)	5932 (3368-9270)	.016
ProGRP (U/L)	38.5 (28.3-65.0)	23.2 (19.5-48.6)	27.2 (22.2-36.5)	.209

Categorical data are presented as frequencies and percentages; continuous variables are presented as mean ± SDs or median (25th-75th quartile) depending on their distribution.
BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; FSGS, focal segmental glomerulosclerosis; HDL-c, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; HT, hypertension; IgAN, IgA nephropathy; LDL-c, low-density lipoprotein cholesterol; MCD, minimal change disease; MGN, membranous glomerulonephritis; NLO, neutrophil to lymphocyte ratio; ProGRP, pro-gastrin-releasing peptide; RAAS, renin angiotensin aldosterone system; SBP, systolic blood pressure.
[a]*P* = .032 compared with score 2.
[b]*P* = .002 compared with score 3 + 4.
[c]*P* < .01 compared with score 2.
[d]*P* < .001 compared to score 3 + 4.
[e]*P* = .027 compared with score 2.
[f]*P* < .001 compared to score 3 + 4.
[g]*P* = .009 compared to score 3 + 4.
[h]*P* = .039 compared to score 3 + 4.
[i]*P* = .016 compared to score 3 + 4.

In another study, GRPR was found to be induced and highly expressed in tubules derived from human biopsies, mouse models, and tubular epithelial cell (TEC) systems. Gastrin-releasing peptide receptor has been shown to contribute to kidney injury and dysfunction by promoting macrophage infiltration, necroptosis, and the autocrine production of GRP. Furthermore, in vivo silencing of GRPR via lentivirus delivery or administration of the novel GRPR antagonist RH-1402 demonstrated

Table 3. Correlation Analysis of Serum Pro-Gastrin-Releasing Peptide and Variables

	Serum ProGRP (n = 49)	
Variables	**r**	***P***
Total cholesterol	0.303	.015
LDL-c	0.313	.012
Proteinuria	0.304	.012
Sodium	−0.059	.633
Potassium	0.067	.586
Calcium	0.298	.013
Phosphorus	−0.108	.378
eGFR	0.099	.422

Significance was determined using Spearman rank correlation coefficient. eGFR, estimated glomerular filtration rate; ProGRP, pro-gastrin-releasing peptide; LDL-c, low-density lipoprotein cholesterol.

Table 4. Correlation Analysis of Variables According to Interstitial Fibrosis/Tubular Atrophy Score

	IFTA Score (n = 49)	
Variables	**r**	***P***
eGFR	−0.675	<.001
Proteinuria	0.415	.003
ESH	0.419	.003
CRP	0.368	.01
Albumin	−0.252	.059
proGRP	−0.202	.168
Sodium	−0.111	.446
Potassium	0.000	1.000
Calcium	−0.239	.098
Phosphorus	0.289	.044

Significance was determined using Spearman rank correlation coefficient. CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; IFTA, interstitial fibrosis tubular atrophy; ProGRP, pro-gastrin-releasing peptide.

significant renoprotective effects, suggesting that GRPR may serve as a potential therapeutic target for preventing kidney injury. Knockout of GRPR protected against kidney injury and inflammation in 2 acute kidney injury (AKI) models induced by ischemia/reperfusion (I/R) and cisplatin; more importantly, conditional knockout of GRPR from TECs had an almost equivalent suppressive effect on the restoration of kidney dysfunction and downregulation of inflammatory factors and chemokines. These results demonstrated that GRPR activation in TECs plays a key role in AKI.[27]

Growing evidence suggests that targeting GRPR may represent a specific and effective therapeutic strategy for AKI associated with kidneyinflammation.[5,20,27] Several synthetic GRPR antagonists previously reported in the literature, such as RH-1402, RC-3095, and Pd176252, have been shown to alleviate kidney injury by reducing inflammation and oxidative stress levels.[5,27] In this study, the fact that kidney biopsies were performed during the early phase when inflammation in tubular cells is more prominent than fibrosis may explain the higher serum proGRP levels observed compared to those in advanced fibrosis. This suggests that early inhibition of GRPR may be important in preventing or slowing the progression of permanent kidney damage. Although serum proGRP levels were higher in patients with an IFTA score of 3+4 compared to those with a score of 2, the increase did not reach statistical significance. This may be related to the relatively small number of patients with IFTA 3+4 in this study, potentially due to the reduced need for kidney biopsy in patients presumed to have advanced-stage kidney fibrosis. It is believed that these findings should be supported by future studies involving a larger number of patients with advanced kidney fibrosis.

There are some limitations associated with the study. Initially, it was conducted at a single center, and the underlying etiologies of kidney pathology among the patients were heterogeneous. Additionally, the number of participants with advanced kidney fibrosis was relatively low, which may have affected the statistical power of the analyses. Therefore, larger-scale studies involving a greater number of patients with advanced fibrosis are warranted. Moreover, due to its design, careful interpretation should be made regarding the cause-and-effect relationship between serum proGRP and proteinuria and kidney fibrosis.

Table 5. Univariate and Multivariate Regression Analyses of Serum ProGRP and Proteinuria Based on the Presence of Interstitial Fibrosis/Tubular Atrophy

	Univariate Analysis				Multivariate Analysis			
Variables	**OR (95% CI)**	**B**	**Wald**	***P***	**OR (95% CI)**	**B**	**Wald**	***P***
proGRP	0.968 (0.936-1.002)	−0.032	3.336	.068	0.967(0.933-1.002)	−0.034	3.517	.061
Proteinuria	1.000(1.000-1.001)	0.00	3.519	.061	1.000(1.000-1.001)	0.00	3.558	.059

OR, odds ratio; ProGRP, pro-gastrin-releasing peptide.

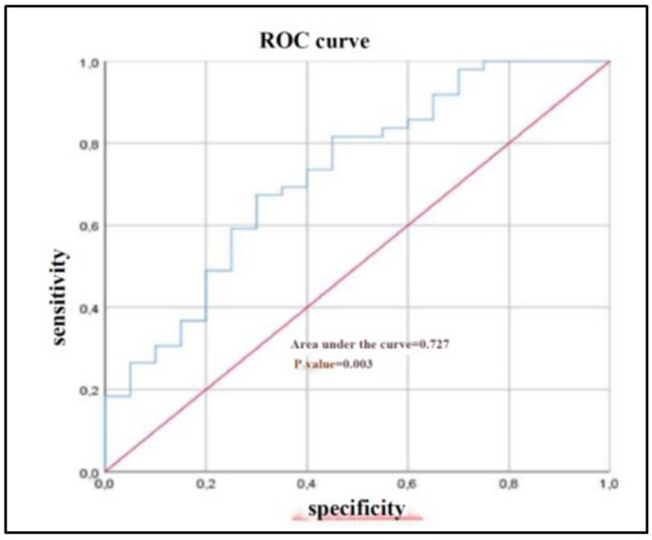

Figure 3. Analysis of the ROC curve of the relationship between serum progastrin-releasing peptide and kidney fibrosis.

In conclusion, based on the findings of this first study investigating the relationship between kidney fibrosis and serum proGRP levels, patients with persistent proteinuria were found to have higher serum proGRP levels compared to healthy controls. It was demonstrated that serum proGRP levels were elevated in the presence of kidney injury and proteinuria. It is believed that these findings provide a basis for the hypothesis that serum proGRP may serve as a predictive biomarker for the presence and severity of acute or chronic kidney injury, and that it may potentially be considered a therapeutic target for the reduction of fibrosis in future comprehensive studies.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Faculty of Medicine, Hitit University (Approval No.: 504; Date: November 29, 2021).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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