Research article

Serum MMP3 Correlates with Renal Function Tests and Insulin Resistance Parameters in Patients with End-stage Renal Disease

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Abstract

End-stage renal disease (ESRD) is associated with changes in serum biomarkers. However, except for the renal function test, there is no definite useful biomarker correlated with the disease and its consequences, such as insulin resistance (IR). In the present study, matrix metalloproteinases-3 (MMP3) and its inhibitor, the tissue inhibitor of metalloproteinase-1 (TIMP1) were examined and correlated with IR parameters. MMP3, TIMP1, renal function tests, and IR parameters were measured in 60 ESRD patients and the results were compared with thirty healthy controls. After controlling for all cofounders, ESRD patients showed a substantial rise in serum MMP3, glucose, insulin, and beta-cell function percentage compared to the control group. While the patients had a lower insulin sensitivity percentage when compared to the controls. TIMP1, insulin/glucose ratio, and insulin resistance index did not vary significantly across groups. MMP3 had a strong relationship with serum creatinine, urea, and eGFR. TIMP1 had a strong relationship with height and weight. High serum MMP3 is associated with increased renal function tests and with changes in the IR parameters in ESRD patients.

1. Introduction

Acute kidney injury (AKI) is one of the worst clinical conditions ever recorded in modern medicine in critically and non-critically ill patients [1]. AKI that continues to be associated with poor outcomes [2]. It can progress into chronic renal and end-stage renal disease (ESRD). ESRD is stage

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five of diabetic nephropathy and is marked by the kidney no longer working properly to meet the needs of body functions [3]. Several comorbidities are usually reported in association with ESRD, namely hypertension, congestive heart failure, and aging-related changes in the immune response, which may aggravate the chronic inflammatory state [4]. Various etiologies are implicated in ESRD, including glomerulonephritis, cystic kidney disease, recurrent kidney infection, chronic obstruction, and secondary hyperparathyroidism, although diabetes mellitus and hypertension are responsible for a large percentage of cases [5]. In addition to renal function tests that changed in ESRD, some biomarkers were found previously to be altered in the disease. The researchers investigated several biomarkers that may be used for diagnosis, prognosis, and follow-up. Two biomarkers that have received relatively less attention in research are matrix metalloproteinases (MMP)-3 and its inhibitor, the tissue inhibitor of metalloproteinase-1 (TIMP1). These parameters play a crucial role in bone resorption, remodeling, and repair [6] in addition to possible effects on the IR state [7]. MMPs are a family of proteins involved in a range of pathologies [8]. MMP3 is an enzyme that is actively involved in joint destruction in rheumatoid arthritis patients and osteoarthritis [9]. It has many functions, plays a crucial role in tumor development [10], and has a protective and anti-tumor function [11]. In human chondrosarcoma-derived cells and articular cartilage, human MMP3 was able to translocate into cellular nuclei and regulate transcription [12]. As the rate of wound healing was shown to be slower in MMP3-deficient mice, it was hypothesized that MMP3 was required for wound closure. A wound's contraction rate may be slowed by MMP3 or by adding a synthetic inhibitor of the MMP family. *In vitro* studies showed that MMP3 promoted endothelial cell proliferation and migration, and suppressed apoptosis, indicating that MMP3 is a regulator of angiogenesis via a non-canonical mechanism [13]. Reducing the production and activity of MMPs has the potential to slow the development of synovial fibrosis by limiting the breakdown of connective tissue matrices [14]. Tissue inhibitors of metalloproteinases (TIMPs) are proteins found in the body that work to keep the rate of matrix degradation and synthesis in equilibrium. TIMP1 is a very effective inhibitor of MMP3 [15]. Some disorders may be linked to an imbalance between MMPs and their related TIMPs [16]. Several disorders, including encephalomyelitis, rheumatoid arthritis, Alzheimer's disease, and cancers, are caused by the dysregulation of tissue remodeling brought on by the overexpression of MMPs or the poor control of MMPs by TIMPs under pathologic settings [17].

Many cell types release TIMPs, including fibroblasts and macrophages, and other cells of the connective tissue, but recent research suggests that T helper cells 1 and 17 are most likely to do so [18]. TIMP-1 inhibits matrix metalloproteinases, but it also has other unique roles, such as enhancing erythroid function and preventing B-cell death [19]. Insulin resistance (IR) is characterized by a reduced physiologic response to insulin stimulation of target tissues, including the liver, muscle, and adipose tissue. Hyperinsulinemia and an increase in beta-cell insulin synthesis occur as compensatory responses to the impaired glucose elimination caused by IR. The metabolic effects of IR include hyperglycemia, hypertension, dyslipidemia, visceral obesity, hyperuricemia, increased inflammatory markers, endothelial dysfunction, and a prothrombic state [20].

The role of the MMP3 and its receptor in the IR state in ESRD patients has not been studied well. The present study aims to study the levels of the MMP3, TIMP1, and IR parameters in ESRD patients after removing the effect of cofounders such as age, BMI, sex, and smoking.

2. Materials and Methods

2.1 Subjects

Sixty ESRD patients (32 men and 28 women) with an average age of 46.45±10.29 years participated in this research. Each patient had a history of AKI that had developed into renal failure and was treated with dialysis. The patients were gathered in the Al-Hakeem General Hospital and Al-Sadr Medical City dialysis units between December 2021 and February 2022 in the Iraqi governorate of Najaf. A thorough medical history that considered the existence of any systemic diseases was used to examine patients. All patients had developed into stage 5 (ESRD) after AKI. The research did not include patients with diabetes, hepatic conditions, and cardiovascular diseases (CVD). A senior physician diagnosed the patients using the tenth version of the "International Statistical Classification of Diseases and Related Health Problems" (2021 ICD-10-CM Diagnosis Code N18.6). All patients received calcium carbonate, epoetin alpha (Eprex®), heparin, and continuous folic acid or folate and iron formula (Fefol®). Thirty healthy people (19 men and 11 women) without observable physical diseases made up the control group. Their ages were comparable with that of patients. All participants signed informed consent forms. All subjects signed an informed document for participation in the study. The Institutional Review Board of the Kufa University protocol was applied (document number 622/2022). Power analysis showed that using an effect size of 0.3, alpha=0.05, power=0.8 and 2 groups, the total sample size should be 90.

2.2 Measurements

Participant venous blood was collected between 8 and 9 a.m., after a 12-h fast. Plain tubes were used to collect venous blood samples. Before the test, samples were aliquoted into three fresh Eppendorf® tubes and kept at -80°C for further analysis. Before the hemodialysis session, serum was taken to evaluate all parameters. ELISA kits provided by Melsin Medical Co., Ltd., Jilin, China, were used to determine the amounts of serum MMP3, insulin, and TIMP1. The kits' inter-assay CV percent was less than 10%, and their sensitivities were under 0.1 ng/mL. Spectrophotometric measurements of glucose, albumin, creatinine, urea, uric acid, inorganic phosphate, and creatinine were made using ready-to-use kits from Biolabo® (Maizy, France). According to the Modification of Diet in Renal Disease (MDRD) study equation [21], the following formula was used to determine the estimated glomerular filtration rate (eGFR):

$$eGFR = 175 \times (S.Cr)^{-1.154} \times (Age)^{-0.203} \times 0.742 \text{ [if female]} \times 1.212 \text{ [if Black]}$$

IR measures, including IR index (HOMA2IR), β-cell activity (HOMA%B), and insulin sensitivity (HOMA%S), were estimated from fasting insulin and fasting blood glucose (FBG) using Homeostatic model assessment (HOMA) and HOMA2 calculator software obtained for free from https://www.dtu.ox.ac.uk/homacalculator/download.php. The input value involves fasting blood glucose and fasting insulin to calculate HOMA2IR, HOMA%S, and HOMA%B.

2.3 Statistical analysis

Analysis of variance (ANOVA) was used to analyze group differences in continuous variables, whereas the χ^2-test was used to assess connections between nominal variables. The correlations between MMP3 and TIMP1 and their ratio to all other biomarkers were determined using Pearson's product-moment correlation coefficient (r) and Spearman's correlation coefficient (rho, ρ). A multivariate general linear model (GLM) was used to examine the correlations between the

measured biomarkers and diagnostic groupings while controlling for potential intervening factors such as sex, age, smoking, height, weight, and BMI. Between-subject effects and partial eta-squared values were used to quantify the correlations between diagnostic classifications and biomarkers. We used the estimated marginal mean (EMM) and standard error (SE) values calculated from GLM and protected pairwise comparisons to arrive at our estimates. Statistical tests were 2-tailed, and a p-value of 0.05 was used for statistical significance. This study used SPSS software version 25, 2017 for statistical analysis.

3. Results and Discussion

3.1 Demographic and clinical data

Table 1 showed that there were no significant differences in height, sex ratio, age, smoking, and family history between ESRD patients and the control group. However, there was a significantly lower body weight and BMI ($p<0.05$) in ESRD patients than in the control group. The patients' disease duration was 2.90 (1.20-5.15) years. In addition to the normal findings in the ESRD disease (increase in urea, creatinine, uric acid, and decrease in eGFR), the first significant finding in the present study was an increase in MMP3 in patients compared with the control group. These results were probably purely due to the ESRD disease because the multivariate GLM analysis removed all the cofounder effects. Previous research showed that after controlling for factors including age, sex, blood pressure, and body mass index, a larger waist-to-hip ratio was linked to a lower GFR, a lower appropriate renal plasma flow, and a higher filtration fraction [22]. Smoking and sex differences also affect many parameters in patients. Therefore, it was necessary to remove the effect of all these cofounders before confirming the decrease or increase of such biomarkers in ESRD disease patients.

Table 1. Demographic and clinical data in ESRD patients and healthy controls

Variables	Control (n=30)	ESRD (n=60)	F/χ^2	df	P
Age year	46.91±6.81	46.26±10.31	0.103	1⁄88	0.749
Sex F/M	11⁄19	28⁄32	2.605	1	0.127
Smoking No/Yes	29⁄1	55⁄5	0.015	1	0.997
Weight kg	74.67±12.98	66.28±10.27	11.451	1⁄88	0.001
Height cm	161.94±11.09	164.95±12.33	1.336	1⁄88	0.251
BMI kg/m^2	28.87±6.42	24.39±3.09	19.826	1⁄88	<0.001
Family history No/Yes	29⁄1	51⁄9	3.722	1	0.082
Duration of Disease yrs.	-	2.90(1.20-5.15)	-	-	-

BMI: Body mass index, Significant difference ($p<0.05$)

3.2 The multivariate GLM analysis

The multivariate GLM and the between-subjects effects tests were used to study the effect of diagnosis on the biomarkers as presented in Table 2.

The multivariate GLM analysis provides the analysis of variance for multiple dependent variables, where each dependent variable may be influenced by one or more component variables or covariates. The population may be segmented into groups based on the factor variables. In Table 2, the diagnosis (being ESRD patients) is the sole significant factor that affects the changes in the

Table 2. Results of the multivariate generalized linear model (GLM*)* analysis and the between-subjects effects of the effect of the diagnosis on the biomarkers

Test	Dependent Variable	Effect	F	P	Partial η^2
Multivariate Tests	All measured parameters	Diagnosis	20.745	<0.001	0.718
		Sex	0.679	0.83	0.129
		Age	1.376	0.171	0.21
		Smoking	0.939	0.543	0.167
		BMI	0.429	0.981	0.088
		Height	0.498	0.957	0.099
		Weight	0.421	0.983	0.086
Tests of Between-Subjects Effects	Diagnosis	eGFR	206.113	<0.001	0.723
		Urea	127.052	<0.001	0.617
		Pi	117.179	<0.001	0.597
		Creatinine	101.567	<0.001	0.562
		HOMA%B	32.766	<0.001	0.293
		Insulin	21.779	<0.001	0.216
		HOMA%S	21.508	<0.001	0.214
		Uric acid	16.363	<0.001	0.172
		MMP3	7.308	0.008	0.085
		Albumin	1.956	0.166	0.024
		TIMP1	0.76	0.386	0.011
		I/G	0.378	0.54	0.005
		HOMA2IR	0.048	0.826	0.001

A significant difference ($p<0.05$)

serum levels of the measured biomarkers with the highest size effect (Partial $\eta^2=0.718$). Other cofounders have no significant effect on the biomarker's levels ($p<0.05$).

According to the between-subjects effect test, the results of which are shown in Table 2, the biomarkers that were significantly affected by the presence of ESRD disease were eGFR (Partial $\eta^2=0.723$), urea (Partial $\eta^2=0.617$), Pi (Partial $\eta^2=0.597$), creatinine (Partial $\eta^2=0.562$), HOMA%B (Partial $\eta^2=0.293$), insulin (Partial $\eta^2=0.216$), HOMA%S (Partial $\eta^2=0.214$), uric acid (Partial $\eta^2=0.172$), and MMP3 (Partial $\eta^2=0.085$). The presence of the disease showed no significant effect on other measured biomarkers (albumin, TIMP1, I/G, and HOMA2IR). The increase in MMP3 in ESRD patients was reported previously [23] and may be due to the role of MMP3 in many biological processes that may affect or be related to the disease. MMPs regulate various cellular functions including apoptosis and angiogenesis, facilitate apoptosis via damaging the mitochondria, and in the later phase, help neovascularization [24]. These MMPs serve an important physiological role, and their abnormal expression or dysregulation is linked to the onset of a wide range of illnesses, including chronic inflammatory conditions, cardiovascular and renal disease, diabetes, neurological disorders, and cancer [25]. Elevated levels of MMP3 in the ESRD patients' group might reflect a gene activation generated by inflammatory cytokines. The increased MMP3 level should be considered a sign of persistent inflammation in the local area [23]. These results are reinforced by the fact that gene expression of MMPs is controlled by cytokines such as interleukin (IL)-6, IL-1β, and tumor necrosis factor-alpha (TNF-α) [26]. Research has shown that increasing kidney damage is linked to the overexpression or improper use of MMPs. MMPs were shown to be higher in

conjunction with glomerular sclerosis [27], as well as chronic kidney disease (CKD). However, the present study shows no significant changes in the TIMP1 in ESRD patients compared with the control group after adjusting for the cofounders. With permission of such cofounders, previous work showed the same lack of significant effect of TIMP1 on ESRD patients with hemodialysis [28].

3.3 Comparison of the biomarkers between groups

The multivariate GLM analysis was made to exclude the effect of recorded cofounders on the levels of the measured biomarkers and to obtain their EMM listed in Table 3.

Table 3. Estimated marginal mean (EMM) and standard errors of the measured parameters, after the exclusion of the effect of the covariates, were obtained from the multivariate GLM analysis.

Variable	Control (n=30)	Patients (n=60)	p-value
S.Cr mg/dL	0.85(0.60)	8.69(0.43)	<0.001
Urea mg/dL	31.11(8.52)	155.87(6.11)	<0.001
Uric acid mg/dL	4.66(0.27)	6.07(0.19)	<0.001
MMP3 ng/mL	50.50(9.50)	83.86(6.82)	0.008
TIMP1 ng/ml	616.58(85.78)	713.71(61.55)	0.386
Glucose mM	5.40(0.17)	5.85(0.12)	0.046
Insulin pM	62.85(3.71)	85.35(2.66)	<0.001
I/G	14.45(0.25)	11.73(0.35)	0.540
HOMA%B	95.92(5.52)	100.33(3.96)	<0.001
HOMA%S	85.96(2.86)	64.68(2.05)	<0.001
HOMA2IR	1.19(0.08)	1.64(0.05)	0.826
Albumin g/L	42.79(1.34)	35.23(0.96)	0.166
eGFR mL/min /1.73 m^2	112.12(4.79)	9.76(3.44)	<0.001

A significant difference ($p<0.05$)

EMM of the measured parameters, after exclusion of the effect of the covariates, showed the usual increase in the renal function tests in ESRD patients compared with the control group. Serum creatinine, urea, and uric acid were significantly higher ($p<0.001$) than the controls, while eGFR was significantly lower than the controls. There were also no statistically significant differences in TIMP1, insulin/glucose ratio, or HOMA2IR levels across the groups. Serum MMP3, glucose, insulin, and HOMA%B are all higher in ESRD patients than in controls ($p<0.05$). In contrast, a decrease in the insulin sensitivity parameter (HOMA%S) was recorded in the patients compared with the controls. The other important finding in the present study was the disturbance in some of the IR parameters in ESRD patients. The increase in serum glucose, insulin, and HOMA%B, in addition to the decrease in the insulin sensitivity parameter (HOMA%S) in patients compared with controls, was related to the abnormal function of beta cells and the abnormal response of the tissues to insulin action. Insulin binds to the insulin receptor on the cell surface to activate a signaling cascade, subsequently promoting glucose transport (glucose influx), glycogen synthesis, lipogenesis, cell proliferation, differentiation, and survival. On the other hand, this cascade leads to the downregulation of gluconeogenesis and lipolysis. The cellular insulin signaling pathway is a complex process consisting of several steps. The kidney plays a significant role in insulin metabolism because it removed 30-80% of insulin from circulation [29].

3.4 Correlation between the major serum biomarkers with all other parameters

The correlations of MMP3, TIMP1, and insulin with all other parameters are presented in Table 4. Serum MMP3 significantly correlates with serum creatinine, urea, and eGFR. TIMP1 has a significant correlation with height and weight. Serum insulin level is significantly correlated with the disease duration, creatinine, urea, glucose, I/G ratio, HOMA%S, and eGFR. While insulin is significantly negatively correlated with weight and HOMA%B.

Table 4. Correlation of MMP3, TIMP1, and insulin with all parameters

Parameters	MMP3	TIMP1	Insulin
Sex	0.059	0.150	-0.096
Age	-0.127	0.095	0.047
Smoking	0.087	0.182	0.130
Family history	0.045	0.099	0.059
Duration of Disease	0.132	0.121	0.412**
Height	0.017	0.267*	-0.097
Weight	-0.024	0.224*	-0.216*
BMI	-0.018	0.000	-0.075
Albumin	0.176	0.017	0.134
Creatinine	0.257*	-0.015	0.497**
Urea	0.269*	0.067	0.454**
Uric acid	0.009	0.111	0.006
MMP3	1.000	0.100	0.089
TIMP1	0.100	1.000	0.015
eGFR	0.311**	0.187	0.402**
Glucose	0.095	-0.008	0.305**
Insulin	0.089	0.015	-
I/G	-0.065	0.013	-
HOMA%B	-0.110	-0.014	-
HOMA%S	0.110	0.014	-
HOMA2 IR	0.153	-0.006	-

* Significant difference ($p<0.05$), ** Significant difference ($p<0.01$)

Multiple factors, including inflammation, oxidative stress, physical inactivity, vitamin D insufficiency, metabolic acidosis, adipokine dysregulation, anemia, and gut microbiome composition, contribute to the development of IR in patients with CKD and patients with ESRD under hemodialysis [30], and IR can exacerbate ESRD [31].

IR is prevalent in ESRD patients [32], and dialysis may partially correct this phenomenon [33]. In patients with ESRD, IR may be attributed to the high incidence of established risk factors, such as obesity, and specific metabolic abnormalities [34]. When CKD is present, inflammation is most often seen in skeletal muscle. Because they primarily represent hepatic abnormalities and CKD inhibits insulin catabolism, estimates of IR from the fasting insulin level may not accurately describe the patients with CKD [34]. In addition, IR in individuals undergoing dialysis has been associated with faster protein catabolism, which may result in a loss of protein energy and malnutrition. IR was not shown to correlate with GFR among CKD patients [35]. However, HOMA-IR showed no significant difference between those with and without CKD [34]. In addition, to the CKD-related causes of IR [36], it has been suggested that the increasing prevalence of well-known risk IR factors including diet, sedentary lifestyle, and obesity, may contribute to the excess IR observed in ESRD [37].

The third important finding was the significant correlation of MMP3 and insulin with serum renal function tests (urea, creatinine, and eGFR). Surprisingly, TIMP1 had a significant correlation with height and weight. Early studies linked the activation of MMP activity to the signaling of insulin or insulin-like growth factor-1 through the PI3K/Akt pathway [38]. Numerous investigations have shown that, depending on the organ, insulin modulates MMPs differently. Boden *et al*. [39] found that in the aortas of rats, free fatty acids and insulin both stimulated the production of pro-inflammatory cytokines, which in turn promoted the activation of MMP2, MMP9, and MT1-MMP. On the other hand, hyperinsulinemia stimulates the degradation of these MMPs [40]. MMPs and IR have a reciprocal relationship in that IR increases the production and activation of MMPs via various signaling pathways, and MMPs contribute to the growth of adipose tissue with a surge in the IR state [41].

It was discovered that MMP3 was elevated in tubular atrophy and interstitial lesions and was negatively linked with mesangial expansion and glomerular damage [42]. TIMP1 was correlated with inflammation in HD patients [43]. However, no difference in IR was found between ESRD patients compared with controls. Other epidemiologic studies [35, 36] indicated that IR is widespread in the early stages of CKD. Lower estimated GFR was linked with higher insulin and HOMA-IR scores among diabetic patients [44]. Furthermore, renal function tests were associated with IR as measured by the higher quartile of the homeostasis model evaluation [45].

4. Conclusions

After adjusting for all cofounders, a significant increase in the serum MMP3, glucose, insulin, and HOMA%B was found in ESRD patients compared with the control group. In contrast, a decrease in the HOMA%S was found in the patients compared with controls. TIMP1, insulin/glucose ratio, and HOMA2IR showed no significant difference between groups. MMP3 shows a significant correlation with serum creatinine, urea, and eGFR. TIMP1 had a significant correlation with height and weight.

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