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Matrix Metalloproteinase 2 as a New Marker for Diagnosis of Chronic Kidney Disease

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ABSTRACT

Background: Chronic kidney disease (CKD) is highly prevalent, irreversible, progressive decrease in renal function. Matrix metalloproteinases (MMPs) have an important role in tissue remodelling by regulating cell death, morphogenesis and wound healing activity. **Objective:** The aim was to assess the diagnostic value of matrix metalloproteinase 2 as a new marker in chronic kidney disease.

Patients and Methods: this study was conducted on (108) individuals who were divided into 3 groups of matching age and sex. Group I (Control group): 36 healthy individuals with normal kidney functions. Group II (CKD group): (stage 1-4) 36 subjects with eGFR between 15-89 ml/min/1.73m^2. Group III (ESRD group): 36 subjects who were under regular hemodialysis for more than 3 months.

Results: There was statistically non-significant difference between the studied groups regarding serum cholesterol. There was statistically significant difference between the studied groups regarding MMP-2. On comparing each two individual groups using Tukey post hoc test for MMP-2, the difference was significant between each two individual groups (MMP-2 was lower in Group I followed by Group II then Group III). The best cutoff of MMP2 in diagnosis of ESRD was ≥0.69615 to <0.78155 with area under curve 0.766, sensitivity 77.8%, specificity 63.9%, positive predictive value (PPV) 68.3%, negative predictive value (NPV) 74.2% and accuracy 70.8%.

Conclusion: There was significantly increased levels of MMP-2 in CKD with more increase in ESRD patients. MMP-2 could be used as a marker in diagnosis of CKD.

Keywords: Chronic Kidney Disease, Matrix Metalloproteinase 2, New Marker.

INTRODUCTION

Chronic kidney disease (CKD) is defined as kidney damage or glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for 3 months or more, irrespective of cause. GFR can be estimated from calibrated serum creatinine (s. Cr) and estimating equations, such as the Modification of Diet in Renal Disease (MDRD) Study equation or the Cockcroft-Gault formula **(1).**

Matrix metalloproteinases (MMPs) are proteolytic enzymes that act on extracellular matrix protein components such as collagens, gelatins, elastins, laminins, fibronectins, and integrins. They are synthesized as zymogens and are activated to functional forms on autoproteolysis or by other proteases **(2).**

MMP2 or gelatinase A is the second member of MMPs family. Its first two substrates were discovered within the components of the extracellular matrix. It was proved that MMP2 overexpression in most if not all tumors were considered a hallmark of cancer aggressiveness **(3).**

The aim was to assess the diagnostic value of matrix metalloproteinase 2 as a new marker in chronic kidney disease.

PATIENTS AND METHODS

A case control study was conducted at Internal Medicine Department, Zagazig University Hospitals and Theodor Bilharz Research Institute from March 2021 to September 2021. Demographic information was collected.

Ethical considerations:

The study was approved by the Ethics Board of Zagazig University and an informed written consent was taken from each participant in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Population of the study:

This study included 108 subjects with matched age groups and of both sexes. ***Participants were classified into three groups:*** **Group I (Control group):** 36 healthy individuals with normal kidney functions, **Group II (CKD group)** (stage 2-4): 36 subjects with eGFR between 15-89 ml/min/1.73m^2, and **Group III (ESRD group):** 36 subjects who were under regular hemodialysis for more than 3 months.

Inclusion Criteria: Age: more than 30 years, CKD or ESRD with more than 3 months duration of expanded hemodialysis (HDx), sex: males and females, and patient consent to enter the study.

Exclusion Criteria: Patients were excluded if they have acute severe infection, malignancies, severe cardiac insufficiency, liver diseases, or acute cerebrovascular accidents, and pregnant females.

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All studied groups were subjected to the following:
Thorough history taking and full clinical examination. Routine investigations to fulfil inclusion and exclusion criteria including: Serum creatinine (Cr), blood urea, complete blood count (CBC), serum cholesterol, and serum triglycerides.

Special investigations: Serum MMP2 by ELISA.
Test principle:

The kit used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assess the level of human MMP2 in samples.

MMP2 was added to monoclonal antibody enzyme well, which was pre-coated with human MMP2 monoclonal antibody that was incubated; then, MMP2 antibodies were labelled with biotin and combined with streptavidin-HRP to form immune complex, then were carried out the incubator and were washed again to remove the uncombined enzyme.

Five mL of venous blood were withdrawn from each subject and was centrifuged and serum was obtained and divided into two portions. The first portion was for kidney function assessment, cholesterol, triglycerides, calcium and phosphorus estimation, which were done on Cobas C 311. The second portion was stored at -20°C until be used for PTH and MMP2 measurement. PTH levels were measured on Cobas e411 autoanalyzer (electrochemiluminescence immunoassay "ECLIA") using kits supplied by Roche diagnostic (Roche diagnostic GmbH, D-68298, Manheim, Germany).

Estimated glomerular filtration rate (GFR MDRD) was calculated using the Modification of Diet in Renal Disease (equation for GFR estimation based on creatinine and patient characteristics).

Statistical analysis

All analyses were performed using Statistical Package for the Social Sciences (SPSS) for windows version 22.0 (SPSS, Chicago, IL, USA). Descriptive data were presented as mean ± SD and range or as frequency and percentages. Chi-square test was used for statistical analysis of categorical variables. Quantitative data were compared by one-way ANOVA test or Kruskal Wallis test. ROC curves were generated by plotting sensitivity vs. (1-specificity) and AUC was calculated and used to assess the predictive value of MMP2 in expecting subclinical atherosclerosis in CKD and ESRD patient. All tests were two-sided, a probability value (P-value) <0.05 was considered statistically significant and P-value < 0.01 was considered statistically highly significant.

RESULTS

There was statistically non-significant difference between the studied groups regarding gender, age or smoking **(Table 1)**.

Table (1): Comparison between the studied groups regarding demographic data

	Group I (Control group)	Group II (CKD group)	Group III (ESRD group)	p
	N=36	N=36	N=36	
Gender:				
Male	16 (44.4)	16 (44.4)	20 (55.6)	0.552
Female	20 (55.6)	20 (55.6)	16 (44.4)	
Smoking:				
Yes	11 (30.6)	12 (33.3)	8 (22.2)	0.555
No	25 (69.4)	24 (66.7)	28 (77.8)	
Age:				
Mean ± SD	49.6 ± 9.39	50.06 ± 12.94	51.13 ± 14.15	0.874
Range	40 – 65	31 – 75	33 – 70	

Each of Group II (CKD group) and Group III (ESRD group) had significantly more cases of hypertension than group 1(Control group) **(Table 2).**

Table (2): Comparison between the studied groups regarding comorbidities

	Group I (Control group)	Group II (CKD group)	Group III (ESRD group)	p	Post-hoc test
	N=36	N=36	N=36		
Diabetes:					
Yes	13 (36.1)	20 (55.6)	11 (30.6)	0.765	
No	23 (63.9)	16 (44.4)	25 (69.4)		
Hypertension:					P_1 <0.001**
No	13 (36.1)	28 (77.8)	30 (83.3)	<0.001**	P_2 0.551
Yes	23 (63.9)	8 (22.2)	6 (16.7)		P_3 <0.001**

Data are presented as number (percentage), P1: the difference between Group I and Group II, P2: the difference between Group 2 and Group III (ESRD group), P3: the difference between Group I and Group III, **: statistically highly significant

There was statistically significant difference between the studied groups regarding hemoglobin, and serum triglycerides. Regarding hemoglobin, the difference was significant between each two individual groups. As for triglycerides, the difference was significant between ESRD and each other group **(Table 3).**

Table (3): Comparison between the studied groups regarding hemoglobin and lipid profile

	Group I (Control group)	**Group II (CKD group)**	**Group III (ESRD group)**	**p**	**Post-hoc test**
	N=36	**N=36**	**N=36**		
Hemoglobin(g/dL): Mean ± SD	12.61 ± 1.86	10.64 ± 0.96	9.05 ± 1.06	<0.001**	P_1 <0.001** P_2 <0.001** P_3 <0.001**
Cholesterol (mg/dL): Mean ± SD	194.5±26.44	195.61±38.85	189.67 ±8.17	0.778	
Triglycerides (mg/dL): Mean ± SD	142.42±6.48	156.17±9.61	219.67±9.04	<0.001**	P_1 0.693 P_2 <0.001** P_3 0.001**

P1: the difference between Group I and Group II, P2: the difference between Group 2 and Group III (ESRD group), P3: the difference between Group I and Group III, *: statistically significant, **: statistically highly significant

There was statistically significant difference between the studied groups regarding MMP-2. The difference was significant between each two individual groups. MMP-2 was lower in Group I followed by Group II then Group III **(Table 4).**

Table (4): Comparison between the studied groups regarding Matrix Metalloproteinase 2

Matrix Metalloproteinase 2	**Group I (Control group)**	**Group II (CKD group)**	**Group III (ESRD group)**	**p**	**Post-hoc test**
	N=36	**N=36**	**N=36**		
MMP2: Mean ± SD	0.573 ± 0.144	0.796 ± 0.128	1.042 ± 0.22	<0.001**	P_1 0.002* P_2 0.001** P_3 <0.001**

P1: the difference between Group I and Group II, P2: the difference between Group 2 and Group III (ESRD group), P3: the difference between Group I and Group III, *: statistically significant, **: statistically highly significant

The best cutoff of MMP2 in diagnosis of ESRD was ≥0.69615 to <0.78155 **(Table 5 and figure 1).**

Table (5): Performance of MMP2 in diagnosis of CKD among the studied participants

Cutoff	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**p**
≥0.69615 to <0.78155	0.766	77.8%	63.9%	68.3%	74.2%	70.8%	<0.001**

**: statistically highly significant

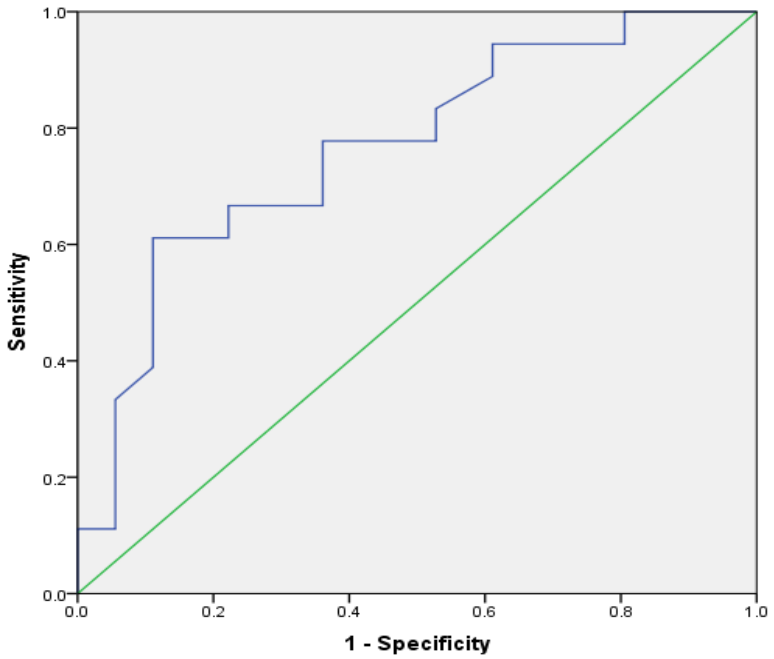

Figure (1) ROC curve showing performance of MMP2 in diagnosis of CKD among the studied participants

DISCUSSION

On comparing each 2 individual groups, the difference was significant between Group I (Control group) and each other group (36.1% of controls have HTN versus 77.8% in Group II (CKD group) and 83.3% in Group III (ESRD group)). This goes with **Gluba-Brzózka *et al.*** [4] who reported that hypertension (HTN) occurred significantly more often in the CKD and ESRD group.

Moreover, **Gluba-Brzózka *et al.*** [5], revealed that beside HTN, DM also could be found much more significantly in CKD group and ESRD group than in controls and attributed these findings to the presence of a high prevalence of a proinflammatory state.

As regard to haemoglobin (Hb) level, we demonstrated that there was statistically significant difference between the 3 studied groups, we noticed that Hb level was lower in Group III (ESRD group) than Group II (CKD group) patients and was higher in controls. This goes in agreement with **Babitt *et al.*** [6], who stated that as kidney disease progresses, anemia increases in prevalence, to the point that it affects nearly all patients with stage 5 CKD and this may be attributed to many factors e.g. erythropoietin deficiency, bone marrow suppression, increased hemolysis, malnutrition and iron deficiency anemia. And this may lead to reduced quality of life, CVD, hospitalizations, cognitive impairment, and mortality.

In our study we noticed that the level of triglycerides (TGS) on comparing the 3 individual groups showed significant increase in ESRD in comparison to the other 2 groups. Unexpectedly, we noticed that there was no significant difference between the studied groups regarding serum cholesterol.

This goes in agreement with **Lee *et al.*** [7] who said that characteristic lipid pattern in CKD patients shows a different profile from the dyslipidemia of the general population, consisting of hypertriglyceridemia, low levels of high-density lipoprotein cholesterol (HDL-c) and variable levels of low-density lipoprotein cholesterol (LDL-c) and total cholesterol (TC) and attributed this to uremia that alter the lipid metabolism, different stages of proteinuria and also the original cause of CKD, which affects lipid metabolism .

The present study showed that there was statistically significant increase in serum MMP-2, in ESRD more than the other 2 groups, moreover we found significant increase in Group II (CKD group) in comparison to Group I (Control group). In accordance with our results, **Elsaeed *et al.*** [8] who revealed significantly higher levels of MMP-2 in patients with CKD in comparison to the Group I (Control group) with higher level in Group III (ESRD group) as compared with the other 2 groups. Our results are in line with the study of **Chen *et al.*** [9] who suggest that MMP2 may be involved in the pathogenesis of atherosclerosis in CKD patients.

Regarding ROC curve for MMP2 in diagnosis of CKD among the studied participants; the best cutoff of MMP2 in diagnosis of ESRD was ≥0.69615 to <0.78155 with area under curve 0.766, sensitivity 77.8%, specificity 63.9%, positive predictive value (PPV) 68.3%, negative predictive value (NPV) 74.2% and accuracy 70.8%. Our results are in consistent with **Kousios *et al.*** [10] who demonstrated that circulating MMPs levels could potentially be of use as biomarkers of adult CKD populations especially MMP-2 which shows the greatest promise.

CONCLUSION

Significantly increased levels of MMP-2 in CKD with more increase in ESRD patients. MMP-2 could be used as a marker in diagnosis of CKD.

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Conflict of interest: Nil.

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