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Investigation of Matrix Metalloproteinase-2 and 9 in Patients with Chronic Renal Failure

Hala Basil Abdulameer[1*], and May Khaleel Ismael[2]
[1,2]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
Corresponding author *

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Abstract

The family of zinc-dependent endopeptidases known as matrix metalloproteinases (MMPs) has been linked to both healthy and unhealthy kidney physiology. Members of the MMP family known as gelatinases (MMP-2, MMP-9) are essential for the degradation of the extracellular matrix (ECM). This effect plays a significant role in the progression of chronic kidney disease (CKD).and as an indicator of chronic renal failure (CRF), which is a permanent loss of kidney function that occurs when the glomerular filtration rate (GFR) falls below 60 ml/min/1.73 m² for more than 3 months. The presented study was established to investigate the MMP-2 and -9 concentrations in the urine and serum among Iraqi patients with CRF undergoing regular weekly dialysis at the Hamida Al-Misfaa dialysis center of Al-Kadhimiyah Educational Hospital as well as the dialysis center of AL Yarmouk Hospital in Baghdad between November 2022 and January 2023. Detection was done using the sandwich enzyme-linked immunosorbent assay technique. The results indicated that MMP-2 and MMP-9 expression levels in both serum and urine were considerably higher in-patient groups compared to healthy individuals.

Keywords: CRF, MMPs, ELISA, detection.

1.Introduction

Chronic renal failure (CRF) is a major public health problem that affects around 10% of the adult population globally. It is characterized by 1) an irreversible in renal function. 2) by a gradual decrease of kidney function, leading to end-stage renal disease (ESRD) (1), when less than 10% of renal function persists; this irreversible loss of renal function results in signs and symptoms known as uremia (2). It is believed that CRF is linked to an increased risk of early mortality has recently gained widespread acceptance in both the medical community and society at large (3). Given that the majority of CRF cases were not clinically recognized, primarily because patients were unaware of CRF risk factors, the global rise in CRF prevalence necessitates improvements in the global strategy for CRF prevention, particularly through the identification of risk factors. Hypertension and diabetes mellitus are the two most prominent risk factors for developing CRF. These two disorders cause vascular changes, which raise the risk of macro and microvascular consequences, including kidney impairment. Hemodialysis and renal replacement therapy is acquired for those with CRF, Hemodialysis is also can be used for those with chronic liver diseases to eliminate toxins that the liver should

normally detoxifies (4). Hemodialysis patients are at risk of contracting viral diseases due to the intrusive nature of therapy and equipment contamination (5). Individuals on hemodialysis are more vulnerable to infections due to compromised immune systems (6).

The Human Matrix Metalloproteinases (MMPs) belong to the subfamily of zinc-dependent endopeptidases that were discovered over 50 years ago. They are a category of 24 endo-proteinases that are released in human body. MMPs are multifunctional enzymes that can cleave extracellular matrix elements such fibrillar and non-fibrillar collagen, proteoglycans, glycoproteins, elastin, and denatured collagen as well as the basement membrane. In addition, in cell surface-associated adhesion and signaling receptors, chemokines, growth factors, cytokines (7). MMPs have proteolytic characteristics and are crucial for a number of physiological and pathological processes, such as tissue remodeling and organ development, control of inflammatory processes, and the development of cancer (8). The connective tissue, vasculature, and nephron compartments of the kidney all contain MMPs. (9), (10). With a few exceptions, MMP levels in normal adult tissues are generally modest, and their secretion levels and effectiveness are unnoticeably regulated. MMPs have long been suspected of contributing to CRF development and progression (11). Gelatinases are thought to play a substantial part in CRF, which is linked to a number of cells signaling pathways, endocytosis, and the emergence of renal inflammation, according to 2017 research. Additionally, their non-proteolytic actions could be directly related to the onset and development of CRF. Studies on gelatinase frequently concentrate on how they typically contribute to ECM hydrolysis. (11). Gelatinase A (72 kDa) known as MMP-2 and gelatinase B (92 kDa) which is MMP-9, are mostly associated with the malignant phenotype of tumor cells. the increasing number of studies shows that circulating MMP-9 and/or MMP-2 levels may be beneficial in assessing prognosis or detecting illnesses. (12). As such, this study aimed to access some ways towards detection and determination of serum and renal MMP-2 and MMP-9 of Iraqi patients undergoing dialysis and to find out CRF association with these parameters.

2.Materials and Methods

The study comprised 60 patients, all of whom suffered from CRF and were undergoing regular weekly dialysis. Blood and urine samples were collected from each patient; 5 ml of the blood were obtained from patients. The blood was transferred into a gel tube and centrifuged at 4000 rpm for 5 minutes to obtain serum and kept frozen at -20 until the time of its use. Urine samples of the studied cases were collected using a disposable, sterile, plastic urine cup from each patient and then transferred to an Eppendorf tube tightly closed by a hinged lid labelled with a special identification number for each sample. Samples were stored at -20 ˚C until immunological examinations were carried out. The samples were collected from patients who attended Hamida Al-Misfaa Dialysis Center of Al-Kadhimiyah Educational Hospital as well as the dialysis center of Al Yarmouk Hospital in Baghdad in Iraq. The collection took place in the time frame between November 2022 and January 2023. The ages of patients ranged between 20 and 84. Thirty (30) samples from apparently healthy individuals were collected as well. The displayed study involved receiving signed consent from each patient after verbally describing what the risks of the procedure were and why the blood sample was being drawn. All patients with hematological malignancies, anemia, and pregnant women were excluded from our research. The hospital's ethics and the College of Science, University of Baghdad Ethics committee approved the research (Ref.: CSEC/0922/0080, Appendix A). Patients were informed of the study's goals and advantages, and they received assurances regarding its risks and rewards. Each patient was asked to fill

out a questionnaire that included general and specific information and if they were in agreement.

2.1.Immunological detection of MMP-2 and MMP-9 by ELISA procedure

Soluble MMP-2 (Catalogue No. SL 1151Hu-1), MMP-9 (Catalogue No. SL 1157Hu), and four kits were used in the study to measure levels in both serum and urine based on the principle of the sandwich, quantitative human Enzyme-Linked immunosorbent assay kit according to the manufacturing company (SunLong Biotech Co., LTD).

2.2The statistical analysis

The Statistical Analysis System (SAS) (2018) was used to detect the effect of different factors in study parameters and quantitative characteristics, including age, disease, and dialysis duration; the mean and standard were calculated. To make a meaningful comparison between means, a t-test was performed. In this study, we used the Chi-square test to compare percentages at 0.05 and 0.01 probability levels. Tables and graphs were used to display each outcome (13).

3.Results

3.1.Distribution of sample study according to Age groups in patients and control

A total of 60 individuals with CRF were divided into three groups. Group 1 contained any patient under the age of 40, which included 12 individuals, accounting for 20% of all samples. Group 2 included all CRF patients ranging in age from 41 to 60 years. This group had 33 patients, accounting for (55%) of the samples. Group 3 included the remaining ages, which included people beyond the age of 60. This group included 15 patients and accounted for 25% of the total samples. According to the statistical analysis, there was a very significant difference between the examined groups (P=0.0084). The age range of patients with CRF was 20–80 years, with a mean of 51.76 ± 1.63, compared to the healthy control group, which was almost similar in age, with a mean of 44.63 ± 2.28; see **Figure 1**.

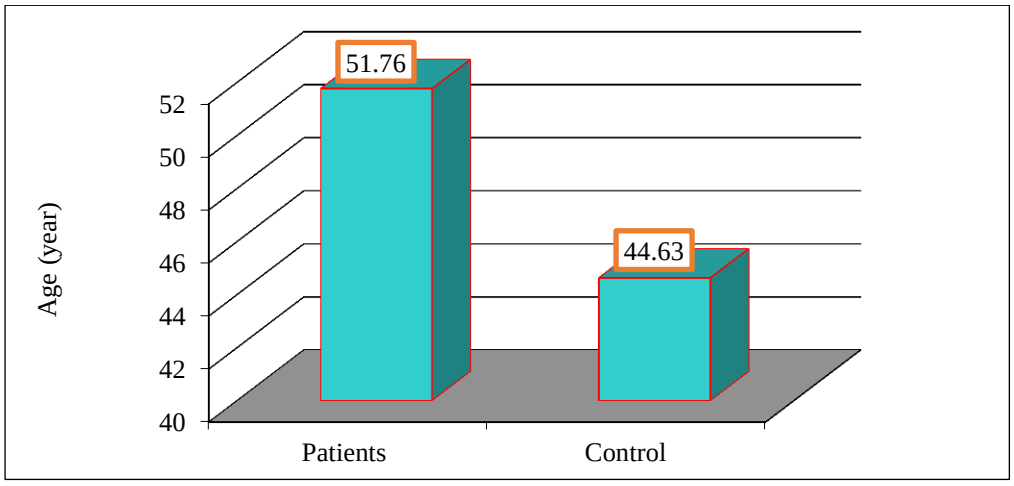

Figure 1. Comparison between CRF patients and control groups in age**.**

3.2.Measurements of MMP-2 and MMP-9 levels

3.2.1. Comparison between study groups in mmp-2 in serum and urine

MMP-2 was found in all of the control group's sera and urine, with levels ranging from 0.74 to 3.36 ng/ml and levels from 0.33 to 2.66 ng/ml, each, with a mean of 1.34 ± 0.11 ng/ml in sera and a mean of 0.975 ± 0.12 ng/ml in urine, respectively. The patient samples that surpassed the cut-off were considered positive. levels ranging from 0.70 to 12.8 ng/ml in sera and levels from 0.26 to 24.9 ng/ml in urine, each with a mean of 2.88 ±0.38 ng/ml in sera and a mean of 2.73 ±0.61 ng/ml in urine, respectively, as shown in **Figures 2 and 3**.

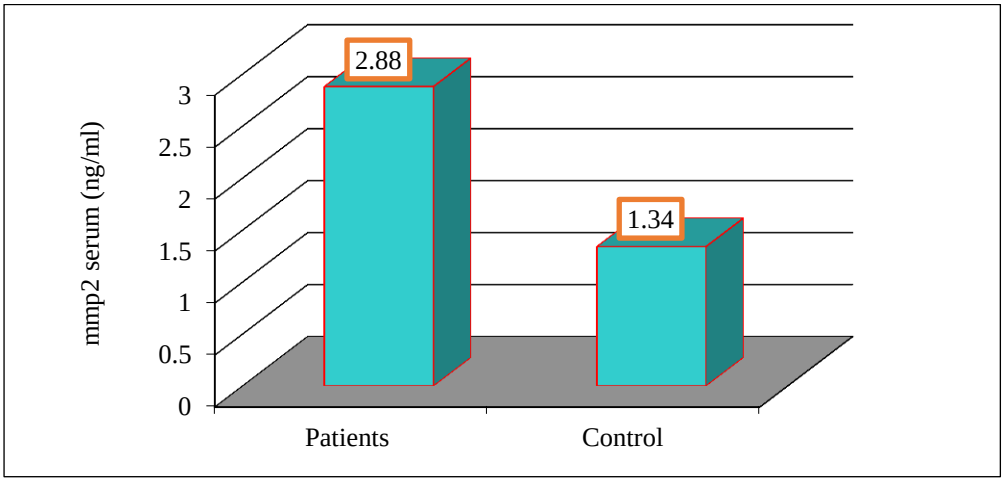

Figure 2. Comparison between CRF patients and control groups in serum mmp-2.

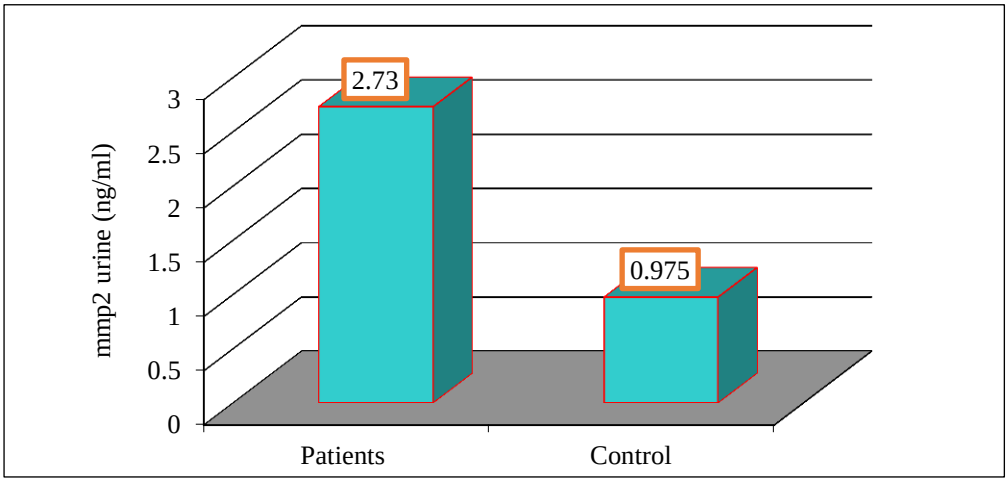

Figure 3. Comparison between CRF patients and control groups in mmp-2 in urine.

3.2.2.Comparison between study groups in mmp-9 in serum and urine

MMP-9 was found in all of the control group's sera and urine, with levels ranging from 6.234 to 19.14 ng/ml and levels from 10.005 to 15.455 ng/ml, each, with a mean of 10.64 ± 0.65 ng/ml in sera and a mean of 11.54 ± 0.27 ng/ml in urine, respectively. Compared to positive patient samples, those with a value greater than the cut-off. levels ranging from 6.455 to 43.25 ng/ml in sera and levels from 10.395 to 63.185 ng/ml in urine, each with a mean of 17.09 ± 1.24 ng/ml in sera and a mean of 21.91 ± 2.12 ng/ml in urine, respectively, as shown in **Figures 4 and 5**.

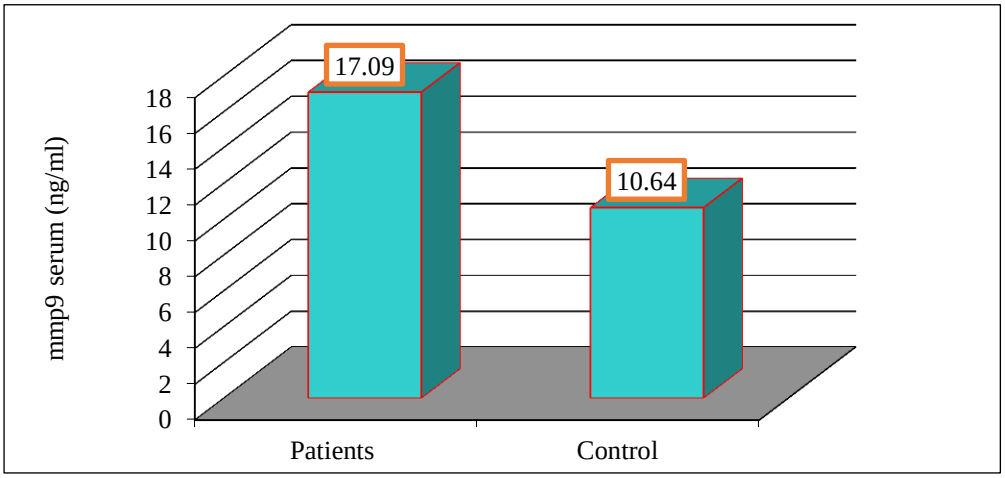

Figure 4. Comparison between CRF patients and control groups in serum mmp-9.

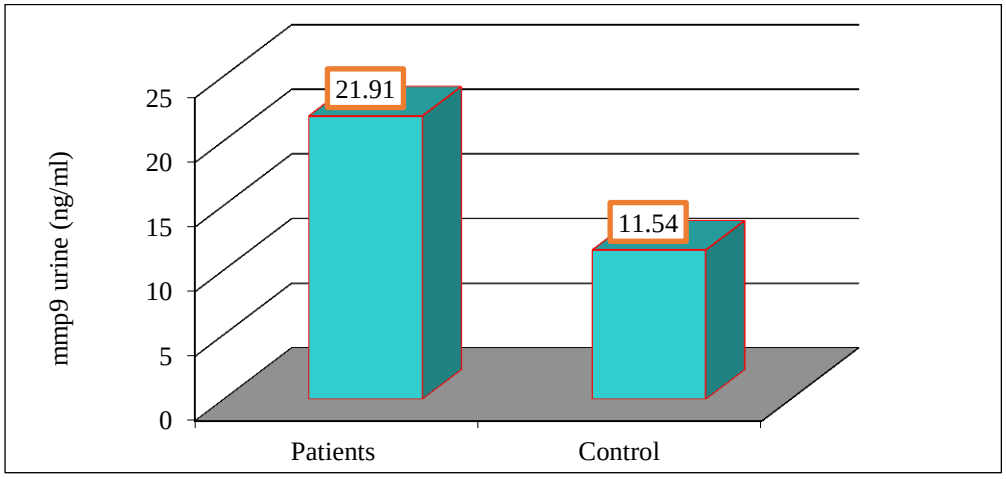

Figure 5. Comparison between CRF patients and control groups in mmp-9 in urine.

4.Discussion

Long-term kidney disease (CKD) is mostly caused by diabetes. CKD is marked by mesangial expansion, glomerulosclerosis, tubular atrophy, and interstitial fibrosis. It has been linked to fibrosis that MMPs are overexpressed and can break down products to make endothelial membrane transition (EMT).

A study revealed that the frequency of CRF in children starts at 1%, rapidly increasing to 5% by 30 years and 20% by 60 years (14). Another study conducted in 2022 revealed that the age group 41-50 years had the highest incidence of chronic renal failure (26%), followed by the age group 51-60 years (24%), and finally, the age group 31-40 years (20%) (15). In this case, the cause could potentially be damage to the kidney nephrons. As nephrons get injured, they lose their function. If the injury continues, more and more nephrons will die. And after a certain age, the surviving nephrons are no longer capable of filtering blood efficiently enough to keep the body healthy. Or because kidneys are aging, kidney function appears to naturally decline with age. It especially affects those people who are aged 75 and more (16).

The current study found a significant increase in MMP-2 concentration levels in sera compared to control levels. The majority of patients with diabetes and albuminuria also had elevated MMP-2 in their urine (17). Researchers have also suggested that the overexpression of MMP-2 could lead to the development of early diabetic nephropathy. Researchers found that 25 kidney samples from patients with type 2 diabetes had higher MMP-2 expression than those from healthy individuals (18). A study observed a reduction in renal MMP-2 secretion in the kidney tissues of early diabetic patients (19). On the other hand, clinical research on human urine revealed significantly higher MMP levels in kidney disease patients compared to healthy volunteers, and a study observed increased serum MMP-2 levels in patients with interstitial fibrosis, kidney transplants, and progressive kidney fibrosis (20). MMP-2 has been linked to the development of fibrosis through stimulating the production of fibroblast growth factor (FGF). As CKD develops, renal interstitial fibrosis decreases, preventing the passage of oxygen and resulting in hypoxia (11). Extracellular volume excess causes interstitial fluid space hypoxia and interstitial injury (21). Increased MMP-2, one of numerous biomarkers enhanced in circumstances characterized by increased inflammation, is considered to represent a distinct risk factor for the development of CKD. In addition, it is possible that MMP-2 and extracellular volume excess share pathophysiological processes in CKD development, such as hypoxia (22). A study in 2012 resulted in the finding that, due to the MMPs proteolytic activity, it was found that it was much higher in DKD patients compared to healthy control participants; urinary MMP activity was (94.5%) in diabetic kidney disease

patients (DKD), as well as (81.5%) and (70%) in diabetic controls and healthy volunteers, respectively. This contrasts with previous findings in renal tissue, where decreased MMP activity has been linked to CKD scarring and fibrosis (23). Polycystic kidney disease (PKD) is the most prevalent hereditary kidney illness. A study revealed a connection between MMP dysregulation and PKD. Serum levels of TIMP-1, MMP-9, and type IV collagen (which MMP-9 targets) are higher in people with PKD than in healthy people (24). Findings from a study done in 2011 showed that MMP-9 seems to play a part in spreading inflammation by creating collagen fragments that attract neutrophils and make them make more MMP-9 (25). Additionally, research suggests that gelatinases could negatively impact the integrity of the kidney basement membrane, primarily composed of type IV collagen and laminin, thereby promoting the development of CRF (26). There are numerous explanations for elevated serum and renal MMP-9 levels in humans; acute post-streptococcal glomerulonephritis correlates with elevated serum MMP-9 and increased neutrophil circulation (27). Furthermore, type 2 diabetes patients showed elevated urine MMP-9 levels (28). Another study resulted in MMP-9 being considerably higher in both non-albuminuric and albuminuric individuals (29). Furthermore, the study found a strong correlation between MMP-9 levels and HbA1c levels, indicating a link with individuals with uncontrolled diabetes. Researchers have found that higher serum and/or urine levels of MMP-2, -9, and TIMPs signal an early stage of renal failure or its progression in persons with CKD, diabetic nephropathy, and following kidney transplantation, although urinary MMP-9 levels in individuals with minimal change disorders were not higher than in controls (30). Therefore, the approval of certain MMPs/TIMPs ratios as prospective early markers of kidney fibrosis in children with chronic glomerulopathies suggests their potential clinical utility (31).

5.Conclusion

Total MMP activity can be easily measured in human serum and urine. Elevated levels of serum and renal MMP-2 and MMP-9 are important factors that worsen kidney function and contribute to the development of chronic renal failure.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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There is no funding for the article.

Ethical Clearance

The study was conducted following the receipt of participant consent and ethical approval from the Ethics Committee of the Biology Department at the University of Baghdad's College of Science (CSEC/0922/0080). This procedure is in line with the guidelines set forth by the Iraqi Ministry of Health and Environment.

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