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Original Research Article

Estimation of Myeloperoxidase and Some Biochemical Parameters in Patients with Chronic Kidney Disease

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Abstract: ***Background*:** Chronic kidney disease (CKD) is a complex disease impacting more than twenty million individuals in the United States. Progression of CKD is associated with a number of serious complications, including increased incidence of cardiovascular disease, hyperlipidemia, anemia and metabolic bone disease. CKD patients should be assessed for the presence of these complications and receive optimal treatment to reduce their morbidity and mortality. A multidisciplinary approach is required to accomplish this goal. ***Aim*:** The aim of the study to estimation myeloperoxidase activity in CKD patients and its association with other biochemical parameters (Creatinine and eGFR). ***Patients and Methods*:** A Case-control study is carried out Baghdad in the Imamian Kadhimian Medical City and Al-Karamah Teaching Hospital between August 2022 and March 2023. The number of CKD male under study was 60 male whose ages were between 25 to 50 years old. In addition, the control group consisted of 30 healthy volunteer males aged between 25 to 50 years and they did not have any diseases. Blood samples collected from each male for measurement of Myeloperoxidase (MPO) by Enzyme linked immunosorbent assay (ELISA), measure of serum creatinine by Abbott c4000 clinical chemistry analyzer and eGFR by MDRD equation. The study showed that the reduced mean level of myeloperoxidase (p value <0.001) in patients group compared with control group, (19.9±6.82 ng/ml) and (42.4±4.98 ng/ml) respectively. Our study revealed that with increasing CKD stage, the myeloperoxidase levels decrease. Also, the elevated mean level of creatinine was 7.47±2.8 mg/dl in patient group was highly significant than control group 0.84±0.158 mg/dl p value <0.001. The decrease mean of GFR was 9.42±3.53 in patients compared with controls 109±22.6 with high statistically significance (p<0.001). The study found a positive correlation between MPO and GFR and negative correlation of serum myeloperoxidase with serum creatinine level in CKD patients.

Keywords: Chronic kidney disease, Myeloperoxidase, serum creatinine & eGFR.

1. INTRODUCTION

Chronic kidney disease (CKD) is recognized as a major health problem affecting approximately 13% of the United States population [1]. Numbers of prevalent CKD patients will continue to rise, reflecting the growing elderly population and increasing numbers of patients with diabetes and hypertension. As numbers of CKD patients increase, primary care practitioners will be confronted with management of the complex medical problems unique to patients with chronic renal impairment. As well documented in the literature, the nephrologist rarely manages the medical needs of CKD patients until renal replacement therapy is required.

CKD is defined as the presence of kidney damage, manifested by abnormal albumin excretion or decreased kidney function, quantified by measured or estimated glomerular filtration rate (GFR), that persists for more than three months [2, 3], leading to loss of normal kidneys capability to eliminate toxic molecules from the body, which detected by renal injury markers, including urinary and hematological alterations. Although creatinine clearances can be calculated from urine creatinine concentration measured in a 24 hour urine collection and a concomitant serum creatinine concentration, a more practical approach in the office is to estimate GFR (estimated GFR or eGFR) from the serum

creatinine concentration, using either the Cockcroft-Gault or the Modification of Diet in Renal Disease (MDRD) Study estimating equations.

Both complications and likelihood of progression to end-stage renal disease requiring renal replacement therapy are more likely to occur in patients with severe CKD. In addition, early intervention will more commonly reduce serious CKD sequelae and slow CKD progression. To facilitate assessment of CKD severity and, the National Kidney Foundation developed criteria, as part of its Kidney Disease Outcomes Quality Initiative (NKF KDOQI™), stratify CKD patients **[**4**]**:

- Stage 1: normal eGFR ≥ 90 mL/min per 1.73 m2 and persistent albuminuria
- Stage 2: eGFR between 60 to 89 mL/min per 1.73 m2
- Stage 3: eGFR between 30 to 59 mL/min per 1.73 m2
- Stage 4: eGFR between 15 to 29 mL/min per 1.73 m2
- Stage 5: eGFR of < 15 mL/min per 1.73 m2 or end-stage renal disease.

Patients with stage 3 or 4 disease progress to end stage renal disease or stage 5 at a rate of 1.5% per year. Stage 1 or 2 CKD patients progress to more advanced stages at approximately 0.5% per year [5]. In addition, the NKF KDOQI provides evidence-based, clinical practice guidelines for all stages of chronic kidney disease to optimize management of related complications.

Myeloperoxidase (MPO) was traditionally considered to be a bactericidal agent [6]. Recent investigations revealed a crucial role of MPO in chronic, nonmicrobial inflammatory processes such as neurodegenerative diseases and athero-sclerosis [7]. MPO, a glycosylated, arginine-rich, extre-mely basic protein (isoelectric point >10) [6] is comprised of two subunits, encoded within a single mRNA. Two of each subunits are assembled with heme molecules to pro-duce the functional enzyme (donor: hydrogen peroxide, oxidoreductase, EC 1.11.1.7). MPO is stored in primary azurophilic granules of leukocytes and the enzyme accounts for up to 5 and 1% of total cell protein content, in neutrophilic polymorphonuclear leukocytes (neutrophils) and monocytes, respectively [8].

The ability of MPO to generate hypochlorous acid/ hypochlorite (HOCl/OCl-) from hydrogen peroxide in the presence of chloride ions is a unique and defining activity for this enzyme [9]. The importance of MPO-catalyzed oxidative reactions and formation of a variety of chlori-nated protein and lipid adducts (with hypochlorous acid as the major oxidant in causing tissue injury by phagocytic cells) has been emphasized [10]. Furthermore, high levels of MPO-mediated endothelial dysfunction may be an important mechanistic link between oxidation, inflamma-tion, and cardiovascular disease (CVD) [11]. An elevated level of plasma MPO served as independent predictor of increased risk of myocardial infarction [12]. However, the role of MPO in chronic kidney disease (CKD) is poorly understood, and not much data is available regarding the variations of this enzyme in these patients. Hence, the present study was undertaken to evaluate the MPO levels in various stages of CKD including end stage renal dis-ease (ESRD). It was speculated that raised levels of this enzyme might be one of the factors responsible for the increased risk that these patients have for developing CVD.

Creatinine is nitrogenous end product of metabolism. Creatinine is the product of muscle creatine catabolism. Creatinine is relatively small molecules (113 daltons) that distribute throughout total body water.

Creatinine formation begins with the transamidination from arginine to glycine to form glycocyamine or guanidoacetic acid (GAA). This reaction occurs primarily in the kidneys, but also in the mucosa of the small intestine and the pancreas. The GAA is transported to the liver where it is methylated by S-adenosyl methionine (SAM) to form creatine.

Creatine enters the circulation, and 90% of it is taken up and stored by muscle tissue. In a reaction catalyzed by creatine phosphokinase (CPK), most of this muscle creatine is phosphorylated to creatine phosphate. Each day, about 2% of these stores is converted nonenzymatically and irreversibly to creatinine [13].

Serum creatinine levels are greatly dependent on dietary intake, total muscle mass, the use of certain medications that can interfere with renal creatinine handling, and renal and extrarenal excretion, all of which might be altered in chronic kidney disease (CKD) [14]. In fact, serum creatinine levels can be insensitive markers of true renal function in CKD. One study assessing the reliability of filtration markers in CKD noted that a >50% reduction in glomerular ultrafiltration needed to occur before the serum creatinine level increased above normal levels (defined as serum creatinine levels of >1.4 mg/dl) [15].

Creatinine is the closest to an ideal endogenous substance for measuring glomerular filtration rate [16]. Creatinine is freely filtered at the glomerulus and is not reabsorbed, but up to 15% is actively secreted by the tubules [17]. Inadvanced renal failure, excretion of creatinine through the gastrointestinal tract increases [18].

The normal serum creatinine (sCr) varies with the subject's body muscle mass and with the technique used to measure it. For the adult male, the normal range is 0.6 to 1.2 mg/dl, or 53 to 106 μmol/L by the kinetic or enzymatic method, and 0.8 to 1.5 mg/dl, or 70 to 133 μmol/L by the older manual Jaffé reaction. For the adult female, with her generally lower muscle mass, the normal range is 0.5 to 1.1 mg/dl, or 44 to 97 μmol/L by the enzymatic method.

Glomerular filtration rate is one of many functions of the kidney. Traditionally, kidney functions are classified as excretory (including glomerular filtration, tubular reabsorption and secretion), endocrine and metabolic. In principle, GFR is the product of the number of nephrons and the average single- nephron GFR. These factors have been investigated extensively in animals, but ascertainment of nephron number and single nephron GFR in humans has been done only in research settings by assessment of glomerular density in biopsy samples [19].

Nephron number is determined during embryonic development, reaching a maximum during the third trimester of pregnancy, and seems to decline with age. The GFR of a single nephron is determined by the actions of haemodynamic factors within glomerular capillaries and the properties of the capillary wall, and is influenced by numerous conditions, such as obesity, hyperglycaemia, use of antihypertensive agents, surfeit or deficit of extracellular fluid, age, sex, pregnancy, dietary protein intake, exercise and diurnal variation. Some evidence suggests that the decline in eGFRcr of approximately 10–40% using the CKD- EPI equation, depending on baseline eGFRcr) is one criterion for acute kidney injury (AKI), a subtype of AKD. Of note, our understanding of the clinical implications of GFR decline, as well as the incidence, prevalence, complications, prognosis, drug dosing and effects of treatment in acute and chronic kidney diseases, is mostly based on studies that have assessed eGFRcr or Scr, rather than measured GFR [20].

2. PATIENTS AND METHODS

A Case-control study is carried out in Baghdad city from August 2022 and March 2023. The numbers of CKD male under study were 60 male whose ages were between 25 to 50 years old. These patients were admitted to the hemodialysis unit and a consultant nephrologist at Imamian Kadhimian Medical City and Al-Karamah Teaching Hospital in Baghdad. Chronic kidney disease was diagnosed based on the presence of the following criteria:

- High levels of urea and creatinine.
- Presence of protein in urine especially albumin that called (proteinuria), and
- Decreased estimated glomerular rate that determine the stages of chronic kidney disease.

In addition, the control group consisted of 30 healthy volunteer males they did not have any diseases aged between 25 to 50 years and were asked to complete a general questionnaire. Patients with metabolic or some disorder diabetes mellitus, viral hepatitis, cancers, whether benign or malignant, who are taking certain medications, and excluded from the study by specific laboratory tests.

The approval permission was submitted to the Director of Health of Baghdad Al-Karkh / Al-Imamin Al-Kadhimin City Teaching Hospital and Al-Karama Teaching Hospital.

3. MATERIALS

In Table 3.1, equipment's and apparatuses used in the current study and in Table 3.2 below, reagent and kits used in the current study.

Table 3.1: Equipment's and apparatuses used in study

Apparatuses	Supplied Company	Origin
Human reader HS	Human	Germany
The ARCHITECT c4000 clinical chemistry analyzer	Abbott	U.S.A
Centrifuge	Hitech	Germany
Micropipette (100-1000 μL)	Slammed	Germany
Micropipette (20-200 μL)	Slammed	Germany
Multichannel Pipettes (10-100 μL)	CappAero	Denmark
Gel tube 10ml	Afco	Jordan
Tips (Blue, Yellow)	Afco	Jordan
Deep freeze	Sanyo	Japan

Table 3.2: Chemical materials and kits used in the study

Material	Company/ Origin	No. of kits
Human Myeloperoxidase ELISA kit	MyBioSource / U.S.A	1
Creatinine	Abbott / U.S.A	1

3.1 Methods

Five ml of blood sample was taken by vein puncture from each subject enrolled in this study. Blood samples were added to gell tubes, after blood clotting, centrifuged at 3000 rpm for 15 minute then clot removed and remain re-centrifuged at 3000 for 10 minute and the obtained serum were aspirated using mechanical micropipette and transferred into clean test tubes which labelled and measurement of Myeloperoxidase (MPO) by Enzyme linked immunosorbent assay (ELISA), and serum creatinine by Abbott c4000 clinical chemistry analyzer.

4. RESULTS

The results in table 4.1 showed the descriptive statistics of age in both study groups. The minimum age was 25 and 28 years in patients and control group respectively. While the maximum age was 52 and 48 years old in patients and control groups respectively. There was no statistical difference between the mean age of patients 40.3±7.53 years and control group 38±6.23 p value =0.117.

Table 4.1: Age characteristics in study groups

Age	Control (n=30)	Patient (n=60)
Mean±SD	38.0±6.23	40.3±7.53
Minimum	28.0	25.0
Maximum	48.0	52.0
P value	0.117^{NS}	

NS: none statistical significance (p>0.05).

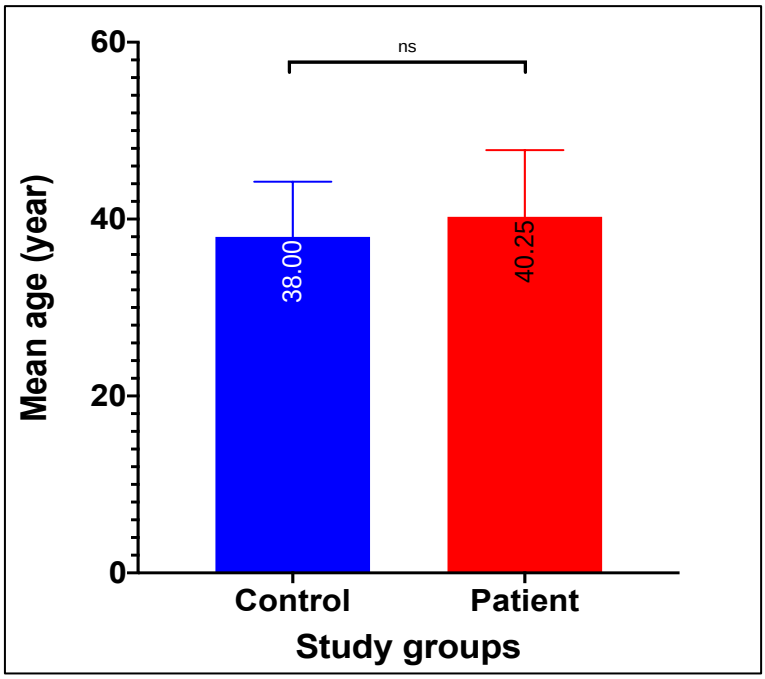

Figure 4.1: Bar-chart represents the age of study groups

The results in table 4.2 showed the reduced mean level of myeloperoxidase (p value <0.001) in patients group compared with control group, (19.9±6.82 ng/ml) and (42.4±4.98 ng/ml) respectively.

Table 4.2: Descriptive analysis of serum myeloperoxidase in study groups

Myeloperoxidase (ng/ml)	Control	Patient
Mean	42.4	19.9
Std. Deviation	4.98	6.82
p value	<0.001**	

**: high statistically significant p≤0.001.

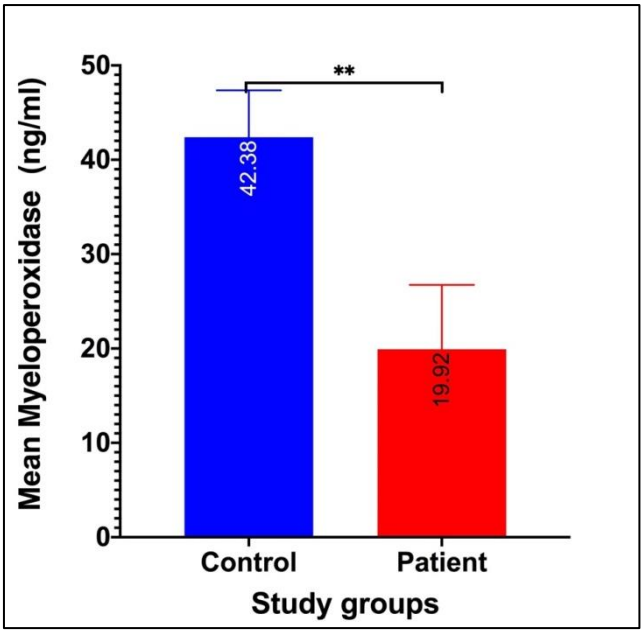

Figure 4.2: Bar-chart represents the serum myeloperoxidase level in study groups

Our study revealed that with increasing CKD stage, the myeloperoxidase levels decrease. As shown in figure (4.2a).

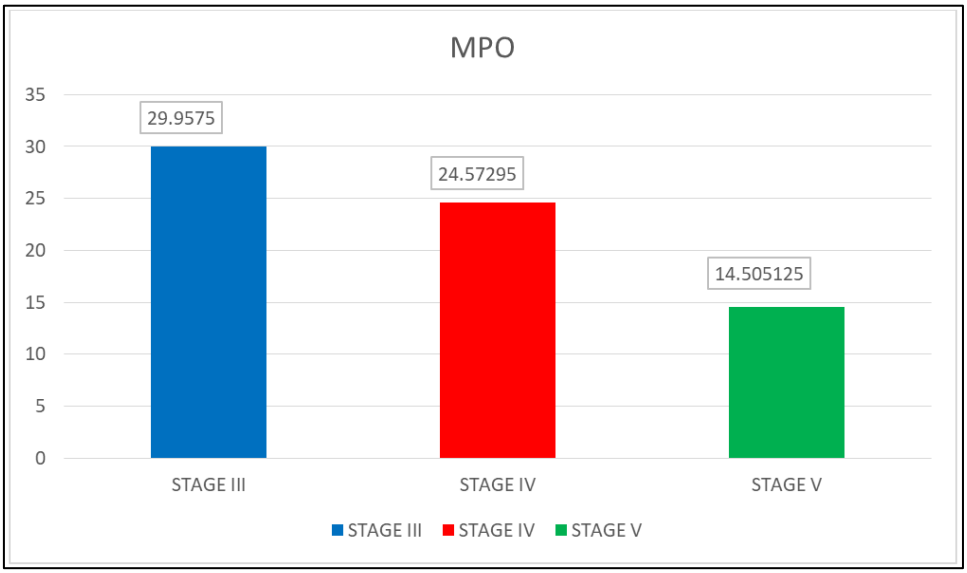

Figure 4.2a: Chart represents the serum myeloperoxidase level in different stages in CKD patients

The results in table 4.3 showed the mean level of creatinine was 7.47±2.8 mg/dl in patient group was highly significant than control group 0.84±0.158 mg/dl p value <0.001.

Table 4.3: Descriptive analysis of serum creatinine in study groups

Creatinine (mg/dl)	Control	Patient
Mean	0.84	7.47
Std. Deviation	0.158	2.8
p value	<0.001**	

**: high statistically significant p≤0.001.

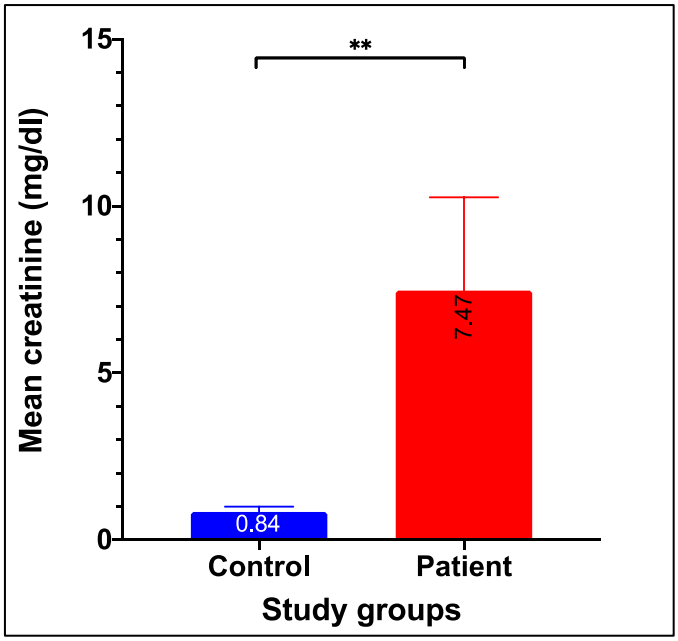

Figure 4.3: Bar-chart represents the creatinine in study groups

The glomerular filtration rate calculated according to MDRD equation were presented in table 4.4. The mean of GFR was 9.42±3.53 in patients compared with controls 109±22.6 with high statistically significance (p<0.001).

Table 4.4: Descriptive analysis of glomerular filtration rate in study groups

eGFR	Control	Patient
Mean	109	9.42
Std. Deviation	22.6	3.53
p value	<0.001**	

**: high statistically significant p≤0.001.

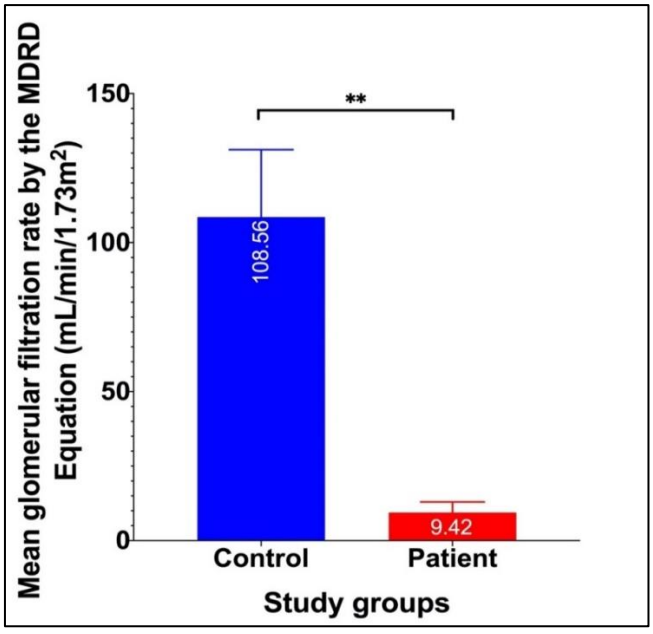

Figure 4.4: Bar-chart represents the glomerular filtration rate in study groups

5. DISCUSSION

Our study included 60 Iraqi patients with chronic kidney disease (males only) with age range 38.0 ± 6.23 years old and 30 people were taken as a control group (males only) and their age was 40.3 ± 7.53 and they did not have any diseases.

Chronic kidney disease, also called chronic kidney failure, involves gradual loss of kidney function. Advanced chronic kidney disease can cause dangerous levels of fluid, electrolytes & wastes to build up in your body.

As there is no cure for chronic kidney disease, the aim of treatment is to help relieve the symptoms & stop it getting worse.

The best way to stop progression of chronic kidney disease into more severe stages is early detection of patients with risk factors to progress.

Myeloperoxidase

Are one of the tests that better to be done in patient with different stages of chronic kidney disease to assess the severity & the predilection for progression into more severe stage of chronic kidney disease.

Our study showed that Myeloperoxidase is significantly reduced in patient with chronic kidney disease and the more severe the stage, the more Myeloperoxidase is decreased, the same result is concluded by *Ahmed, Ahmed Mohammed, et al.,* [21] *and AFSHINNIA, Farsad, et al.,* [22].

Myeloperoxidase levels decrease as the chronic kidney disease stage increases & the least level of Myeloperoxidase was found in stage V.

Also, patients with a level of Myeloperoxidase equal to 10 ± 1 were found to be in more need for hemodialysis & as we informed by the doctors, they need medical consultation by cardiologist & two of them develop cerebrovascular accident (CVA).

So, our study conducted that patients with low levels of Myeloperoxidase are in need for frequent dialysis & they are prone for complication more than other patient with higher levels of Myeloperoxidase.

CORREA, Simon, et al., [23] found that myeloperoxidase levels were higher in patients with chronic kidney disease.

The reason for this difference in results could be attributed to:

- Greater sample size.
- Higher concentrations were more likely women.
- Different ethnicity.

Our study also concluded the serum creatinine level is increased in patient with chronic kidney disease, this came in agreement with *AMIN, Noor, et al.,* [24] and *KAMAL, Azra, et al.,* [25].

6. CONCLUSIONS

Chronic kidney disease is a global disease could affect any age & it associated with high morbidity & mortality. Myeloperoxidase is decreased in patients with CKD, the more higher the stage, the more decrease in MPO level. CKD associated with decreasing eGFR & increasing serum creatinine.

COMPLIANCE WITH ETHICAL STANDARDS

Before starting the study, all participants received an explanation of the procedure and the risks they would face later in their participation, and provided informed consent to participate in this study. The study was approved by the Ethics Committee of Baghdad/Karkh Health Department, and all procedures were in accordance with the Declaration of Helsinki.

CONFLICT OF INTEREST

The authors state no conflicts of interest regarding the research, authorship, and/or publication of this article.

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