



MYELOPEROXIDASE IN CHRONIC KIDNEY DISEASE

Biochemistry

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ABSTRACT

Background: Many authors have demonstrated the presence of MPO containing cells as well as MPO proteins and its activity in renal diseases. Various studies have also implicated a role of MPO in pathogenesis of Cardio Vascular Disease (CVD). There are evidences that suggest that patients suffering from CKD are at increased risk for CVD. Hence, serum level of MPO was determined in healthy controls as well as patient diagnosed with CKD which were categorized according to the level of kidney disease I, II, III, IV and V including End Stage Renal Disease (ESRD). **Objective:** To study serum myeloperoxidase level in different stages of chronic kidney disease. **Methods:** For 1 year level of serum MPO was estimated by sandwich ELISA method in 30 control subjects and 30 cases diagnosed with CKD. Patients with underlying malignancy, SLE, systemic vasculitis and congenital heart disease were excluded. Further, the cases were categorized into 5 stages based on their GFR calculated by Gault Cockcroft formula. The statistical analysis of differences of means for baseline characteristics and serum MPO levels between cases and controls was done by using parametric test (unpaired 't' test) for quantitative analysis. Between-group comparisons were performed using 1-way analysis of variance (ANOVA). **Results:** On comparison of the level of serum MPO (values in Mean±SD) between the cases (18.661±13.088) and control subjects (61.761±24.840) a highly significant difference was noted (P-value ≤0.01). A highly significant difference was also found on comparison of the levels of serum MPO (values in Mean±SD) in various stages of CKD [Stage 1 (n=6) 103.422±7.043], [Stage 2 (n=6) 26.862±2.136], [Stage 3 (n=6) 14.698±3.796], [Stage 4 (n=6) 9.325±1.717] and [Stage 5 (n=6) 4.068±1.302] in the cases (P-value ≤0.01). **Conclusion:** There was rapid decline in serum MPO level with advancing chronic renal failure, which appeared to be caused by a synergism of different mechanism such as inflammation, oxidative stress and genetic components.

KEYWORDS

Myeloperoxidase, chronic kidney disease

INTRODUCTION

Numerous line of evidence implicates a role of Myeloperoxidase (MPO) in the pathogenesis of cardiovascular disease (CVD). It is a well-accepted fact that patients with chronic kidney disease (CKD) are at increased risk for CVD. MPO is a pro-oxidant enzyme that could involve in increased susceptibility of these patients with CVD¹. Myeloperoxidase (MPO), is a heme containing, cationic, and glycosylated protein with a molecular weight of approximately 144 kD, abundantly prestored in azurophilic granules of polymorphonuclear leukocytes (PMNLs) and, in negligible amount, monocytes, and macrophages² in the kidney, monocytes represent the major source of MPO in lipid-associated glomerulopathy, a process very similar to lipid-associated development of atherosclerosis. MPO is associated extracellularly and intracellularly with monocytes/macrophages in different stages of lesion severity³. Chronic kidney disease is a progressive syndrome in which the kidney loses its ability to filter blood, concentrate urine, excrete wastes, and maintain electrolyte balance. Chronic kidney disease is a slowly progressive disease that can occur over many years or decades (4). End stage renal disease (ESRD) is the end result of many forms of CKD. It is characterized by severely limited kidney function that is insufficient to sustain life.

CKD can be classified into following stages

- **Stage 1:** Kidney damage with normal or increased GFR, GFR ≥ 90 mL/min/1.73m²
- **Stage 2:** Kidney damage with mildly decreased GFR, GFR of 60–89 mL/min/1.73m²
- **Stage 3:** Moderately decreased GFR, GFR of 30–59 mL/min/1.73m²
- **Stage 4:** Severely decreased GFR, GFR of 15–29 mL/min/1.73m²
- **Stage 5:** GFR < 15 mL/min/1.73m², or receiving dialysis⁵.

AIMS AND OBJECTIVES

To study serum myeloperoxidase level in different stages chronic kidney disease.

MATERIALS AND METHODS

The study was conducted over a period of 12 months. Subjects were recruited from patients presenting in department of medicine (Nephrology), with a primary diagnosis of kidney dysfunction after obtaining written informed consent from the patient and Ethical clearance from the Institutional Human Ethics Committee.

Study Design

- Type of the study: Observational and Analytical.
- Sample size and Sampling method: Minimum number of 60 subjects. Convenient sampling.

Selection of Subject
Control Group (n = 30)

Inclusion Criteria

- Chronic kidney disease patients with GFR > 60ml/m

Exclusion Criteria

- Malnourished Subjects

Experimental Group (n = 30)

Inclusion criteria

- Patients more than 10 years of age diagnosed having chronic kidney disease.
- GFR < 60ml/m

Exclusion Criteria

- Under lying active malignancy, systemic lupus erythematosus, systemic vasculitis, congenital heart disease.

METHODOLOGY

Peripheral venous blood sample were collected under basal condition with aseptic precautions. The blood samples were collected in a plain yellow top vacutainer for estimation of serum MPO levels. The freshly collected sample were centrifuged for 15 minutes at 3500rpm within an hour of collection and stored at -20°. The analysis of blood urea nitrogen (BUN), creatinine and phosphorus was done on DxC

800, fully automated clinical chemistry analyzer marketed by Beckman and Coulter, India (Pvt) Ltd. Serum Myeloperoxidase was estimated by a kit provided by EUROIMMUN, Medizinische Labordiagnostika AG, US. Myeloperoxidase in the samples was estimated by a sandwich enzyme linked immunosorbent assay (ELISA), based on principle of competitive binding. The ELISA test provides a quantitative in vitro assay for human autoantibodies of IgG class against myeloperoxidase (MPO) in serum.

RESULT

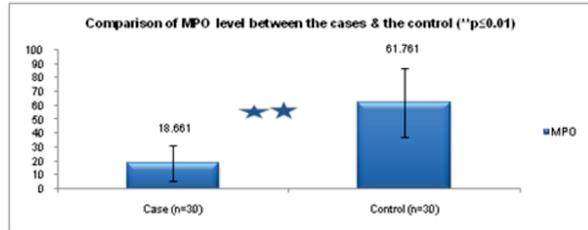


Figure 1: Bar diagram showing the comparison of MPO level between the cases and the control

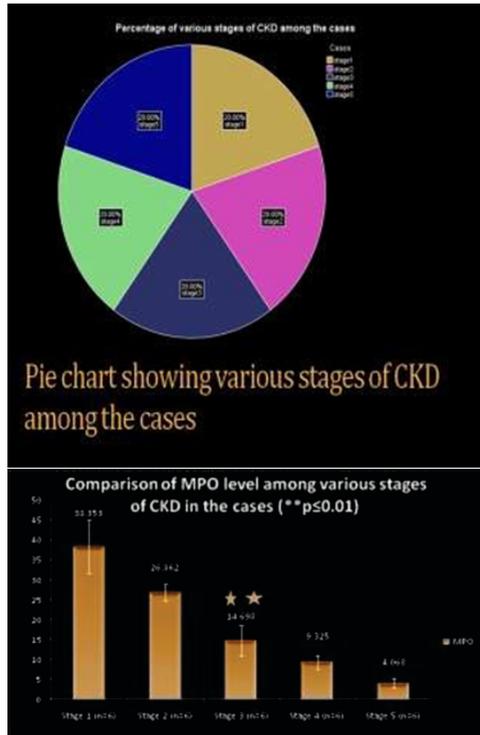


Figure 2: Bar diagram showing comparison of MPO level among various stages of CKD in the cases

DISCUSSION

Myeloperoxidase (MPO) is an enzyme that has been seen to play an important role in initiation and progression of atherosclerosis. Several mechanisms by which elevated level of MPO can promote cardiovascular complication has been described⁶. In their paper Pecoits-Filho et al showed that a higher expression polymorphism in the promoter region of MPO is associated with cardiovascular disease in ESRD patients⁷. Cardiovascular disease is the leading cause of death in ESRD. CVD often begins well before the onset of end stage of chronic renal disease and is associated with recognizable risk factors. In the general population, CVD morbidity and mortality have declined substantially over the past three decades through risk factors identification and reduction and more effective treatment of CVD. Several studies have indicated that MPO serves as an enzymatic source for bioactive lipids and its generated adduct products have been shown to adversely affect the protective action of high-density lipoprotein (HDL) as well as to induce endothelial dysfunction^{8,9}. These observations thus emphasized the role of other agents in causing increased oxidative stress mediated endothelial dysfunction, which might manifest in higher incidence of cardiovascular complication in CKD. The reasons for the fall in the levels of plasma MPO are not known with certainty. However, it could be speculated that this may be

due to the uremic environment. Cape-illere et al¹⁰ suggested that in uremic patients plasma protein could be directly oxidized by an MPO independent mechanism that leads to generation of advanced oxidation protein products that occurs mainly via an MPO independent pathway. Cyanate (CNO⁻) is chemically similar to thiocyanate (SCN⁻), a so-called pseudohalide, which can serve as substrate for peroxidases such as lactoperoxidase, MPO and eosinophil peroxidase yielding hypothiocyanous acid (HOSCN)¹¹. Dirnhuber and Schutz¹² reported that under physiological conditions, cyanate (CNO⁻) forms spontaneously in solutions containing urea, and is present in urine and the body fluids of uremic patients and reaches equilibrium in the molar ratio of approximately 0.0075:1 (CNO⁻-urea). Therefore it is possible that CNO⁻ also may act as a pseudohalide but that the product of an MPO-CNO⁻ reaction, if any, could be relatively impotent in bacterial killing and/ or may directly inhibit MPO¹³. Hence, MPO measurement may be useful in cardiac risk stratification especially in dialysis patients who have a larger burden of atherosclerosis cardiovascular disease than the general population. However, very few studies have examined the role of MPO in patients with ESRD and their outcome. Therefore, this study was performed to determine MPO activity in patients with CKD. However, results have shown that serum MPO levels are significantly low in CKD patients as compared to controls. The present study of CKD patients was undertaken with a view to confirm that serum MPO levels would be decreased with progression of CKD including ESRD. A total of 60 subjects were studied which were divided into two groups of 30 each. One group consisted of normal controls and second group consisted of CKD patients.

Serum MPO level in Healthy Subjects

The age group of normal subjects was between 17 to 80 years. Male constituted 33.33% (n = 10) of this group while females were 66.67% (n = 20). Level of MPO varied from 24.84 - 89.84 RU/ ml. A. Madhusudhana Rao et al¹⁴ conducted a similar study on 100 subjects (73 subjects were male and 27 female in age group of 19-78 years), where control subject (n = 20) were taken having normal kidney function with GFR > 90 ml/min/1.73m² and serum MPO was calculated in controls as well as cases in different stages of CKD by spectrophotometric method using 0- dianisidine (sigma Aldrich) and hydrogen peroxide was calculated and the level of serum MPO came as 217± 31.6 (mean±SD). Our value of the controls using ELISA method was 61.761±24.840 with maximum and minimum value being 97.270 and 22.460.

Comparison between cases and controls

In our study the level of serum MPO (RU/ml) in cases was found to be 18.661 ± 13.088 with minimum and maximum value being 24.84 and 97.27. In comparison of these parameter in controls (n = 30) and cases (n=30) the difference was found to be statistically significant p value<0.01.

Level of Serum MPO in different stages of CKD in cases

The study conducted by A. Madhusudhana Rao et al (14) revealed that there is significant decrease in the level of serum MPO as the CKD progresses.

Table 1:

Study	Controls	Stage I	Stage II	Stage III	Stage IV	Stage V
Madhusudhana et al	217±31.6 (n=20)		100±5.6 (n=20)	73±9.1 (n=20)	44±8.8 (n=20)	21±6.16 (n=20)
Our Study	61.8±24.8 (n=30)	38.4±6.7 (n=6)	26.7±2.1 (n=6)	14.7±3.8 (n=6)	9.3±1.7 (n=6)	4.1±1.3 (n=6)

Values are Mean±SD

This supports our study that states, the level of serum MPO declines with advancing stage of CKD. Our study was found to be statistically highly significant as p-value<0.01

The study conducted by A. Madhusudhana Rao et al revealed that there is significant decrease in the value of GFR (ml/min/1.73 m²) as the CKD progresses into different stages.

Value of GFR in different stages of CKD in cases

Table 2:

Study	Controls	Stage I	Stage II	Stage III	Stage IV	Stage V
Madhusudhana et al	113±8.6 (n=20)		71±8.8 (n=20)	38±6.3 (n=20)	18±3.1 (n=20)	11±2.4 (n=20)
Our Study	133±96.4 (n=30)	103±7.0 (n=6)	73±4.5 (n=6)	38±8.4 (n=6)	18±2.0 (n=6)	8±3.9 (n=6)

Values are Mean±SD

This supports our study that states, the value of GFR declines with advancing stage of CKD. Our study was found to be statistically highly significant, p-value ≤0.01.

CONCLUSION

In our study there was high prevalence of atherosclerotic cardiovascular disease in patients with chronic kidney disease including ESRD. We also found that there was a strong independent correlation between serum MPO level and stages of CKD, the level was found lowest in stage 4 and stage 5, emphasizing that MPO plays a mechanistic role in pathogenesis of endothelial dysfunction leading to cardiovascular disease in these patients. According, to our study the serum MPO level in CKD cases was found to be (18.7 ± 13.1) , which was lower than the healthy subjects (61.8 ± 24.8) . Keeping in view significant difference in serum MPO level of healthy and in patients diagnosed with CKD, it is fairly reasonable to accept the importance of serum MPO as an indicator of progression of CKD stages including ESRD. The test was found to be highly significant ($p \leq 0.01$) in determining the level of serum MPO in different stages of CKD in the cases. Hence, predicting progression of CKD to ESRD. In conclusion, our study has shown that, serum MPO levels decline as the CKD progresses, possibly due to inhibitory effect of uremic toxins on the enzyme. It is suggested that serum MPO may be used as a important tool for diagnosing unrecognizable clinical risks in this population.

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