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*Original Research Article*

# Evaluation of Myeloperoxidase in Saudi Patients with Chronic Renal Failure

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## ABSTRACT

**Objective:** Evaluation of Myeloperoxidase (MPO) enzyme in Saudi patients with chronic renal failure (CRF), compared with control healthy subjects.
**Back ground:** The patients with renal failure are at a risk for cardiovascular diseases (CVD), MPO is a pro-oxidant enzyme, and through its strongest oxidative capacity suggested have a role in protection against CVD. In this study the level of MPO enzyme was expected to decrease in patients with CRF compared with control healthy subjects which mean that they were more vulnerable to CVD.
**Methods:** A case control investigated of MPO enzyme and renal function tests (urea and creatinine) levels in renal failure patients compared with a healthy subjects. Plasma MPO was assayed by a spectrophotometric method. Serum urea and creatinine were estimated on a clinical chemistry analyzer using standard laboratory procedures.
**Results:** The mean MPO level was significantly decreased in CRF patients compared to control healthy subjects (t=-23.737) (99% confidence intervals and t-test-23.737 significant at $P < 0.01$). There were significant positive correlation between MPO and Glomerular Filtration Ratio (GFR) (r=0.657, $P < 0.01$) and significant inverse correlation with Urea (r=-.729, $P < 0.01$) and Creatinine (r=-0.772, $P < 0.01$). No correlations observed in control healthy subjects ($P = 0.97$).
**Conclusion:** The level of MPO was significantly decreased in patients with CRF, this decreased may be due to inhibition of uremic toxins.

***Keywords:*** Oxidative stress, Uraemic toxins, Cardiovascular disease (CVD).

## INTRODUCTION

Myeloperoxidase (MPO) is a hemoprotein found in the azurophilic granules of neutrophils and, to a lower extent, in monocytes and macrophages and is involved mainly in innate immune defense and bactericidal activity. In addition, MPO functions as a major enzymatic catalyst for

the initiation of lipid peroxidation, a pivotal process in atherogenesis, at sites of inflammation. MPO serves as a catalytic sink for nitric oxide, decreases nitric oxide bioavailability, and impairs nitric oxide–dependent vasodilatation. Plasma MPO level predicts nitric oxide–dependent flow-mediated dilatation in humans. In immunohistochemical studies, MPO and products of MPO-catalyzed oxidation reactions have been identified in human atherosclerotic lesions. [1]

Myeloperoxidase (MPO), the most abundant protein in Neutrophils (also found in Monocytes), is the focus of inflammatory pathologies. Most recent work has indicated that it is an excellent biomarker for human cardiovascular risk. Its ability to catalyze reaction between chloride and hydrogen peroxide ($H_2O_2$) to form hypochlorous acid is unique among mammalian enzymes and is considered to be the dominant function of MPO in vivo. Hypochlorous acid is a powerful antimicrobial agent, and extremely reactive with biological molecules causing much of the damage mediated by Neutrophils in inflammatory diseases. MPO also exhibits peroxidase activity that catalyzes oxidation of a number of substrates by ($H_2O_2$). This activity has been widely used to assess the amount of MPO. Unfortunately, its specificity is very poor for unpurified biological samples due to the presence of other peroxidases. Peroxidases however, generally do not produce hypochlorous acid; the only exception is Eosinophil peroxidase that produces hypochlorous acid at pH levels below 5. The chlorination activity of MPO has a pH optimum of near neutral pH. Therefore, assay conditions can be set so to provide for MPO enzyme specificity. [2]

Many evidences indicate a role of myeloperoxidase (MPO) in the atherogenesis of cardiovascular diseases (CVD). The patients with chronic Renal Failure (CRF) are at a risk for CVD, MPO is a pro-oxidant enzyme that should be involved in the increased susceptibility of these patients to CVD. So the level of MPO enzyme was measurements in renal failure patients and compared with control healthy subjects, MPO level determined by spectrophotometric methods. [3]

The ability of MPO to generate hypochlorite (HOCl/OCl−) from hydrogen peroxide in the presence of chloride ions is a unique and defining activity for this enzyme. [4] The importance of MPO-catalyzed oxidative reactions and formation of a variety of chlorinated protein and lipid adducts (with hypochlorous acid as the major oxidant in causing tissue injury by phagocytic cells) has been emphasized.[5] Furthermore, high levels of MPO-mediated endothelial dysfunction may be an important mechanistic link between oxidation, inflammation, and cardiovascular disease (CVD).[6] An elevated level of plasma MPO served as independent predictor of increased risk of myocardial infarction. [7]

## MATERIALS AND METHODS

***Subjects:*** The subjects were a group of Saudi patients suffer from CRF attended to the prince Abdurrahman Alsedairy hospital (Aljouf, Saudi Arabia) from the period since August 2012– January 2013, a total of 60 patients with CRF (36 male and 24 female, aged 25–67 years) were involved in this study, who attended for treatment and follow up in kidney clinic. Control subjects were twenty volunteers subjects (13 male and 7 female, aged 24–60 years) with normal renal function had GFR > 90 ml/min/1.73 $m^2$.

Sample size was calculated for unmatched case-control study, using Open Epi version 3.03.17, with a power 80%, alpha 0.05, the range of cases by Fleiss to Fleiss CC(continuity correction) from 57 – 69; and control from 19 – 23. Then we

collect 60 patients and 20 controls in a systematic random sample. [8]

***Ethical considerations:*** The objectives of the study were explained to all individuals participating in this study, an informed consent was obtained from all participants with the questionnaire. Ethical approval was obtained from the Health Policy and Management Health Research Ethics from the local health authorities (prince Abdurrahman Alsedairy hospital).

***Laboratory methods:***

Blood samples were collected from the study groups (patients and controls) (3ml) in plain containers using disposable syringes. All blood samples were allowed to clot at room temperature and then centrifuged at 4000 R.P.M to obtain the serum. The samples were centrifuged for 15 min at 2000 rpm and stored at −20°C until analysis. Serum urea and Creatinine was determined spectrophotometrically using commercial kits from Human medical company.

GFR was calculated according to MDRD (Modification of Diet in Renal Disease Study) equation. [9]

GFR=175Xstandarized $Scr^{-1.154}$X$age^{-0.203}$X1.212(if black)X0.742(if female)

MPO activity was assayed spectrophotometrically using o-dianisidine (Sigma-Aldrich) and hydrogen peroxide. [10] In the presence of H2O2 as oxidizing agent, MPO catalyses the oxidation of o-dianisidine yielding a brown coloured product, oxidized o-dianisidine, with a maximum absorbance at 470 nm, according to the following overall reaction:

**$2H_2O_2$ + *0*-dianisidine → oxidized dianisidine + $4H_2O$.**

One unit (U) of MPO activity was defined as that degrading 1 μmol of hydrogen peroxide per minute at 25°C.

***Statistical analysis:***

A comparison of continuous variables (MPO, Urea, Creatinine and GFR ) between two patients and control samples was performed by using independent samples t-test, and we used correlation coefficient and path analysis and constructed a causal model to carry out the relationships between variables, SPSS version 19 and AMOS version 18 was used to perform this analysis. Moreover, $p < 0.01$ was considered statistically significant.

## RESULTS

The mean level of MPO was decreased significantly in patients compared to controls (t=-23.737, p<0.01). The mean level of urea and Creatinine was significantly decreased in patients than controls (t=6.113, t=5.971 p<0.01) respectively. Also GFR mean level was significantly decreased in patients compared to controls (t=-7.691, p<0.01) (Table 1).

Table 1: Comparison of clinical features of patients and controls, according to biochemical measurements (GFR, Urea, Creatinine and MPO), represents Means, Standard deviations and 99% confidence intervals for two samples control and patients.

| | Parameter | Mean | SD | Lower | Upper |
|---|---|---|---|---|---|
| Control | MPO | 206.05 | 9.91 | 199.71 | 212.39 |
| | Urea | 24.65 | 7.78 | 19.67 | 29.63 |
| | Creatinine | 0.88 | 0.16 | 0.77 | 0.98 |
| | GFR | 118.45 | 9.63 | 112.29 | 124.61 |
| Patients | MPO | 80.23 | 22.92 | 72.35 | 88.11 |
| | Urea | 59.68 | 25.14 | 51.05 | 68.32 |
| | Creatinine | 2.67 | 1.24 | 2.21 | 3.13 |
| | GFR | 64.32 | 30.86 | 53.72 | 74.92 |

There were insignificant correlations among the variables of control samples, there were insignificant correlations (P > 0.01) between MPO and urea (r=0.172), MPO and Creatinine (r=0.418) and MPO and GFR (r=0.009). In contrast, there were insignificant inverse correlation between Urea and Creatinine (r=-0.078), Urea and GFR (r=-0.173) and Creatinine and GFR (r=-0.219) (Table 2).

Table 2: Simple correlations among the variables of Control sample.

| Variables | MPO | Urea | Creatinine | GFR |
|---|---|---|---|---|
| MPO | 1 | | | |
| Urea | 0.172 | 1 | | |
| Creatinine | 0.418 | -0.078 | 1 | |
| GFR | 0.009 | -0.173 | -0.219 | 1 |

Note that all the correlation coefficients are insignificant

Table 3: Simple correlations among the variables of patients.

| Variables | MPO | Urea | Creatinine | GFR |
|---|---|---|---|---|
| MPO | 1 | | | |
| Urea | -0.729** | 1 | | |
| Creatinine | -0.772** | 0.709** | 1 | |
| GFR | 0.657** | -0.630** | -0.685** | 1 |

** Significant at P < 0.01 .

In the variables of patients, there were significant inverse correlations (P < 0.01) between MPO and urea (r=-0.729) and MPO and Creatinine (r=-0.772). In contrast there were significant inverse correlations (P < 0.01) between GFR and urea (r=-0.630), and GFR and Creatinine (r=-.685). There were a significant correlation (P < 0.01) between MPO and GFR (r=0.657) (Table 3).

The causal model in (Figure 1) explains that Urea, Creatinine and GFR are individually effect on the MPO. It is also explained that GFR effects on Urea and Creatinine (Figure 1).

All relationships between variables of control are not significant. The results of the causal model in Figure 3 explained statistically significant relationship between MPO with Urea, Creatinine and GFR. And GFR with Urea and Creatinine beyond the 0.01 level (Figure 2).

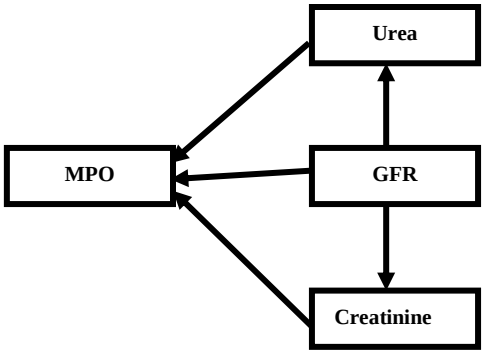


**Figure 1: Path diagram for a causal model.**

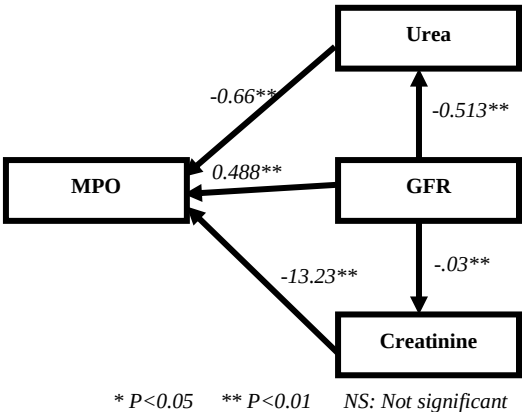


**Figure 3: The results of the causal model for patients.**

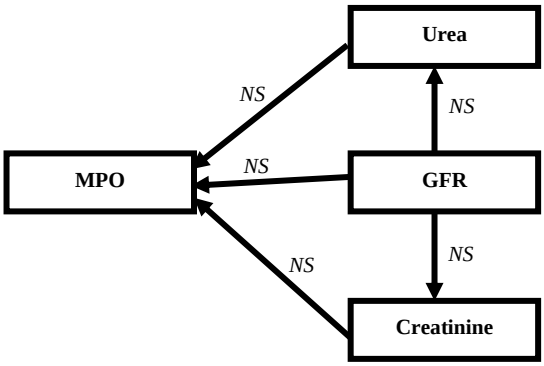


**Figure 2: The results of the causal model for Control samples.**

## DISCUSSION

The results of the study documented significant decreased in the mean level of MPO in patients with CRF. Recent studies have emphasized the importance of myeloperoxidase for cardiovascular disease. [7,11-14] Patients with renal diseases are at a higher risk for CVD; Other studies indicated several mechanisms by which MPO level was a useful marker to predicted the risk of coronary artery disease and cardiovascular events. [11,15] Therefore, this study determined MPO activity in patients with chronic renal failure. According to results

(table 1) the mean level of MPO was significantly decreased in CRF patients compared to controls. So the low level of this enzyme indicated risk for patients with cardiovascular disease.

Myeloperoxidase also likely serves as a marker for general capacity for oxidative damage to the vasculature rather than functioning solely to directly consuming nitric oxide.[16] However damage by oxidative stress and inflammation lead to endothelial dysfunction and this role of MPO in these patients. The study indicated opposite of finding of Capeillere *et al*, [17] because he found that MPO level in renal failure patients does not arise so it have not a significant role in oxidative stress mediated endothelial dysfunction, because he suggested that in renal failure patients, MPO oxidize plasma proteins and the generation of oxidation protein products occurs mainly via an MPO independent pathway. So that many agents other than MPO could increased oxidative stress endothelial dysfunction, which may lead to incidence of cardiovascular complications.

The cause for the decrease MPO level is not known but many studies [3,18,19] reported that possible mechanism interpretation this decreased in level, it could be speculated that this may be due to the uremic environment. 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).[18] Under physiological conditions, cyanate (CNO−) forms spontaneously in solutions containing urea, and is present in urine and the body fluids of uraemic patients and reaches equilibrium in the molar ratio of approximately 0.0075:1 (CNO−-urea).[19] We supported that according to our finding (table 3, figure 3), as in figure 3, in which the causal model explained that there were statistically significant relationship between MPO with Urea, Creatinine and GFR. And GFR with Urea and Creatinine beyond the 0.01 level. It means that when urea and Creatinine increased one unit the MPO decreased 0.66 and 13.23 unit respectively. And when GFR increased one unit the MPO increased 0.488 units. Moreover, when GFR increased one unit, Urea and Creatinine decreased 0.513 and 0.03 respectively.

## CONCLUSION

The study concluded that the level of MPO was significantly decreased in patients with renal failure; this decrease may be due to inhibition of uraemic toxins to the enzyme activity.

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