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Evaluating the Efficacy of Myeloperoxidase and other Biochemical Parameters in the Diagnosis of Chronic Kidney Disease among Diabetic Patients: A Cross-sectional Study

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Abstract

Introduction: Chronic kidney disease (CKD) is a public health problem across the globe which is characterized by the accumulation of various substances like urea, creatinine, electrolytes, water, in the human body. CKD patients are more prone to increased risk of developing oxidative stress due to metabolic disorders, immunodeficiency and persistent infections and inflammation. Myeloperoxidase may participate as one of the mediators of oxidative modification of biomolecules/tissues and contribute to the development of co-morbidities and complications in patients with CKD.

Aims and objectives: The present study was undertaken to assess the role of Myeloperoxidase, HbA1c, Urea, Creatinine urine microalbumin and eGFR in chronic kidney disease in diabetic and non diabetic patients.

Materials and Method: A cross sectional study consisting of two groups with 50 participants each was carried out. Group-I included 50 Chronic kidney disease patients without Diabetes mellitus (CKD-ND) & Group-II included 50 patients of Chronic kidney disease with Diabetes mellitus (CKD-DM). Myeloperoxidase, HbA1c, Urea, Creatinine and urine microalbumin were estimated in the blood and urine samples by using Erba & ELISA kits on XL-640 clinical chemistry analyzer and ELISA reader.

Results: The values of Myeloperoxidase were statistically decreased in diabetic patients with chronic kidney disease(Group-II)when compared with Non diabetic patients with chronic kidney disease (Group-I). Myeloperoxidase levels were compared with Urea, Creatinine, Microalbumin and eGFR levels. eGFR levels showed a significant negative correlation with MPO levels.

Conclusion: The present study showed that decreased serum MPO can be used as an indicator for chronic kidney disease in diabetic patients which can prevent further complications. MPO levels decline steadily as CKD progresses, which might be due to the inhibitory effect of uraemic toxins on the enzyme.

Keywords*: Myeloperoxidase, Chronic Kidney Disease, Diabetes mellitus, Estimated glomerular filtration rate (eGFR), urine Microalbumin.*

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Introduction

CKD is a slow and progressive disorder of kidney dysfunction which is reported worldwide affecting 750 million people globally [1]. In India, diabetes and hypertension account for 40–60% cases of CKD [2]. Chronic kidney disease (CKD) is a global threat to

health in general and for developing countries like India, because of the dietary habits, socio economic status and life style.

CKD patients are at higher risk related to oxidative stress, metabolic disorders and other pathologies. Hence CKD is a major cause of morbidity and mortality due to lack of proper diagnosis and treatment. The mammalian heme peroxidase enzymes play a major role in human immune abnormalities. Heme peroxidases are the acceptors which utilize H_2O_2 to catalyze oxidative reactions. MPO is an oxidizing agent whose elevated levels have been associated with CAD, atherosclerotic lesions. Hence this study is first of its kind to correlate the association between MPO levels in CKD with and without diabetes.

MPO is produced by neutrophilic granulocytes which along with heme (as co-factors) is microbicidal/ bactericidal by producing HOCl, H_2O_2 and Cl^- anions. MPO is a basic arginine rich glycosylated protein (isoelectric point >10) [3] which is comprised of two subunits, encoded within a single mRNA molecule. Studies also revealed that apart from being bactericidal its major role also has been associated with non-microbial inflammatory process and neuro degenerative diseases. There are studies which correlate MPO as enzymatic source of bioactive lipids and other products which have emphasized that they adversely affect the cardio protective capacity of high density lipoproteins and as well induce endothelial dysfunction [4,5]. This study is an attempt to understand the role of MPO as a predictor for early detection of CKD in diabetic patients. Hence a cross-sectional study was carried out among CKD patients with and without diabetes. The following are the objectives :

1).Correlation of MPO levels with eGFR

2). Correlation of MPO levels with Microalbumin

Materials and Method

A cross sectional study was conducted in the department of Biochemistry in collaboration with Medicine and Nephrology, at central laboratory of Prathima Institute of Medical Sciences during the period of June 2015 to September 2016. Two comparative groups consisting of 50 study participants each were enrolled in the study. The study was approved by the ethical committee of the institute (IEC/PIMS: 2015/01). Age and sex matched healthy controls were enrolled from the allied hospital. Informed consent was obtained from all the participants in the study. Clinical history and demographic details of the patients were collected using structured questionnaire. In each group, subjects were selected by simple random sampling technique. The groups were divided as follows:

Group-I 50 CKD without Diabetes (CKD-ND)

Group-II 50 CKD with Diabetes (CKD-DM)

Inclusion criteria:

Age ≥ 40 years, and ≤ 70 years

Clinically Confirmed diabetic patients with duration of diabetes >5 years and <10 years

Clinically confirmed patients of CKD

Exclusion criteria:

Not willing to consent,

Duration of diabetes <5 years and > 10 years

Under aseptic conditions, 5ml random venous blood sample was collected from each participant by anti cubital vein puncture in a disposable syringe of which 2 ml was collected into a EDTA vaccutainer for the estimation of HbA1c and the remaining was transferred into a plain bulb. After centrifugation serum was separated and used for the estimation of biochemical parameters like Urea, Creatinine and Myeloperoxidase. A random urine sample of 5 ml was collected in a sterile container for the estimation of Microalbumin. Myeloperoxidase was estimated by ELISA method. Urea and Creatinine were estimated in serum sample and Microalbumin was estimated in the urine sample collected from the above subjects on XL-640fully automated analyser. eGFR was calculated using CKD-EPI formula.

Serum Urea is estimated by Berthelot method..[6]

GFR is calculated by using CKD-EPI creatinine equation 2009.

$GFR = 141 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black][7].

Glycosylated haemoglobin is a boronate affinity which was estimated by immuno chromatographic method using Nycocard reader.

Creatinine was estimated by Jaffe's Method. [8] Microalbumin is estimated by pyrogallol red method [9]. Myeloperoxidase is estimated by standard protocol using ELISA technique [10].

Statistical analysis: The results were expressed as Mean±SD and student't' test was done to compare the mean parameters. Correlation analysis were done using Karl Pearson's correlation coefficient. Statistical significance was considered at the level of 5% (p-value < 0.05). Statistical analysis was performed by SPSS version 20 .

Results

The results of two groups were expressed as Mean±SD. Table1 shows the comparison of Group-I (CKD-ND) with Group-II (CKD-DM). It summarizes the Mean±SD of Serum Myeloperoxidase, Serum Creatinine, Urea, HbA1c,Urine microalbumin and eGFR. The Myeloperoxidase levels of Group-II were (7.59±3.71) which is significantly lower as compared to in Group-I (10.41±4.75). The eGFR levels were significantly decreased in Group-I (15.40±12.21) compared to Group-II (19.14±13.51) respectively. Microalbumin levels were higher in Group-II (138.78±90.47) when compared to Group-I (71.94±64.26). The Creatinine levels of Group-I were (6.24±4.13) as compared to (4.78±2.77) in Group-II. The Urea levels of Group-I were (84.86±40.76) as compared to (81.08±41.79) in Group-II. The values of HbA1c in Group-II (7.69±1.17) were higher when compared to Group-I (5.34±0.49). The results also reveals that the values of serum Myeloperoxidase were significantly decreased in Group-II when compared to Group-I. 'p' value(<0.001)was found statistically significant for Myeloperoxidase and Creatinine in Group-II.

Table 1: Comparison of CKD-ND and CKD-DM

Parameters	**Group-I (CKD-ND) n=50 Mean± SD**	**Group-II (CKD-DM) n=50 Mean± SD**	**'p'- value**
MPO ng/ml	10.41±4.75 (4.7-22.5)	7.59±3.71 (4.3-19.9)	<0.001
eGFR (ml/min/1.732 m^2)	15.40±12.21 (03-49)	19.14±13.51 (03-47)	<0.001
Microalbumin mg/L	71.94±64.26 (11.6-256.1)	138.78±90.47 (23-400)	<0.001
Creatinine(mg/dl)	6.24±4.13 (1.5-21.4)	4.78±2.77 (1.5-13.3)	<0.001
Urea(mg/dl)	84.86±40.76 (32-212)	81.08±41.79 (21-169)	<0.001
HbA1c%	5.34±0.49 (4.3-6.1)	7.69±1.17 (6.4-11.2)	<0.001

Correlation studies revealed a negative correlation between Myeloperoxidase and eGFR in Group-I (CKD-ND) and a positive correlation in Group-II (CKD-DM). The correlation co-efficient value 'r' was -0.005and 'p' value was 0.972 for Group-I (CKD-ND). The value of 'r' was 0.114 and 'p' value was 0.431 in Group-II (CKD-DM). This indicates that there was a significant positive correlation between Myeloperoxidase and eGFR in Group-II when compared with Group-I. The reason for non significant correlation may be due to non linearity and small sample size.

Table 2: Correlation between eGFR, Myeloperoxidase and Microalbumin

Groups	Correlation Coefficent	r	'p' value
Non Diabetic	eGFR & MPO	-0.005	0.972
	eGFR & Microalbumin	-0.348	0.013*
	MPO & Microalbumin	-0.136	0.345
Diabetic	eGFR & MPO	0.114	0.431
	eGFR & Microalbumin	-0.401	0.003*
	MPO & Microalbumin	-0.274	0.054

Note:*significant with 5% level of significance

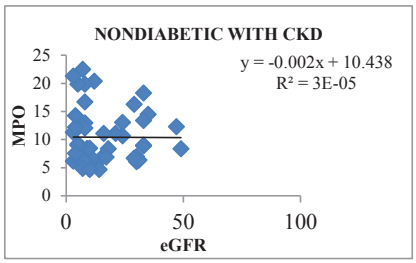

Figure1: CKD-ND (Group-I)

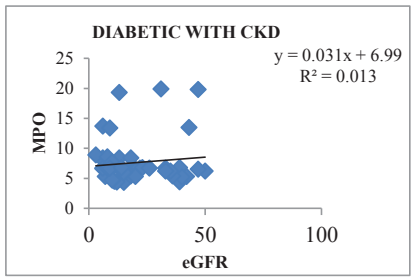

Figure 2: CKD-DM (Group-II)

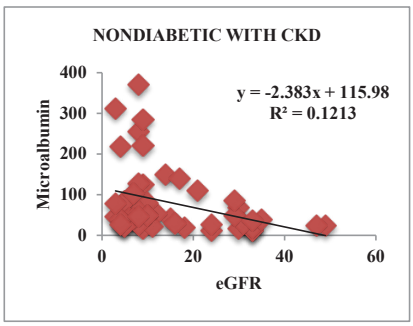

Figure 3: CKD-ND (Group-I)

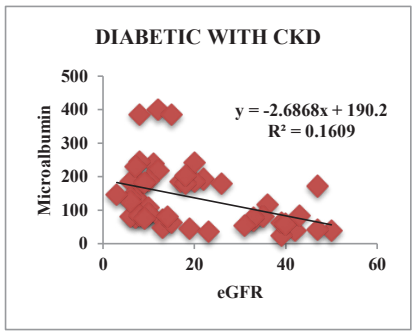

Figure 4: CKD-DM (Group-II)

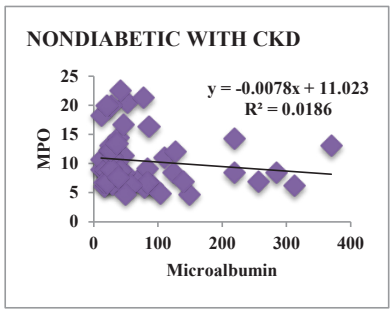

Figure 5: CKD-ND (Group-I)

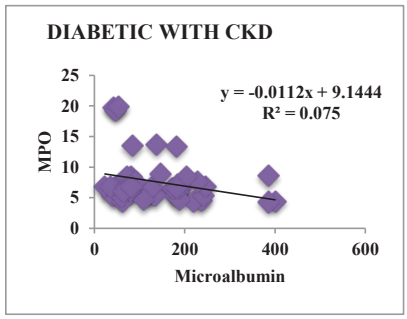

Figure 6: CKD-DM (Group-II)

Discussion

Proteinuria is the common indication for end stage renal diseases (ESRD) which is more commonly seen in patients with CKD. It might contribute to the complications associated with the progression of the disease. It also influences the mortality in CKD patients. HOCl and MPO derived oxidants induces damage to the renal tissue there by contributing to the renal complications [11]. Lipid peroxidation is induced

by extracellular MPO which is capable of catalyzing lipoprotein peroxidation in vivo, thus resulting in atherosclerosis which is common in CKD patients. Due to its cationic character it can bind to the negatively charged structures of endothelial cells and albumin [12].

In this study, we aimed at evaluating the levels of Myeloperoxidase, Microalbumin and calculation of eGFR using CKD-EPI equation in diabetic and non diabetic patients. There was a significant decrease in MPO levels, and an increase in microalbumin levels in Diabetics with CKD when compared with non diabetics with CKD.The decrease in MPO levels implies that MPO and its derived oxidants such as HOCl (hypochlorous acid) interferes with various cell functions which may contribute to damage of renal tissues resulting in the accumulation of uremic toxins which indicates decline in renal function.

Our study demonstrates that there is a progressive fall in mean serum MPO levels with advancing renal disease. MPO is an enzyme which has been shown to play an important role in the initiation and progression of atherosclerosis. Several mechanisms by which elevated levels of MPO can promote cardiovascular complications have been described [13]. Therefore this study was undertaken to determine the activity of MPO in patients with CKD. However, our results have shown that serum MPO levels are significantly lower in CKD patients with diabetes mellitus as compared to CKD patients without diabetes mellitus. The present study has also shown a significant negative correlation between Urea and MPO in CKD-DM; while no significant correlation was observed in the CKD-ND subjects. Hence, it could be speculated that the decline in MPO levels in CKD patients might be due to the inhibitory action of uraemic toxins, particularly CNO-, on this enzyme.

Conclusion

Estimation of serum MPO in CKD patients can be helpful in better prognosis. This study shows that serum MPO levels can be used an indicator for CKD patients with Diabetes mellitus in assessing the renal impairment and to prevent further complications. Microalbuminuria should be corrected at an early stage to delay the renal damage and development of cardiovascular complications in CKD patients.

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Conflict of Interest: None

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