DOI: 10.7860/JCDR/2015/12532.6222

Original Article

Biochemistry Section

Emerging Role of Myeloperoxidase in the Prognosis of Nephrotic Syndrome Patients Before and After Steroid Therapy

SREELATHA SOUPARNIKA[1], BENEDICTA D'SOUZA[2], VIVIAN D'SOUZA[3], SUSHANTH KUMAR[4], POORNIMA MANJREKAR[5], MANOHAR BAIRY[6], RAJEEVALOCHANA PARTHASARATHY[7], SRINIVAS KOSURU[8]

ABSTRACT

Background: Myeloperoxidase (MPO) is a myelocyte derived iron containing enzyme particularly involved in host defense by destroying foreign micro organisms invading the body. Numerous evidences suggest that MPO is involved in the pathogenesis of many inflammatory diseases, especially atherosclerosis.

Aim: Present study deals with the role of MPO in the renal function and progression of disease in Nephrotic syndrome patients.

Study Design and Settings: Case- Control Study carried out in Kasturba Medical College Hospital, Mangalore, India.

Materials and Methods: Forty newly diagnosed Nephrotic syndrome cases, 40 age and sex matched healthy controls and 15 subjects in Nephrotic syndrome remission, were included in the study. Myeloperoxidase enzyme was assayed by 4 amino antipyrine methods in all the subjects. Other renal parameters like urea, creatinine, Blood Urea Nitrogen (BUN), BUN- Creatinine ratio (BUN/Cr) total protein, albumin, globulin, albumin – globulin ratio (A/G ratio) and estimated Glomerular Filtration Rate (eGFR) were also analysed. 24 hour urine protein-creatinine ratio was estimated in Nephrotic syndrome cases and remission group by turbidimetric assay.

Statistical Analysis: Students paired t-test and Wilcoxon Signed Rank test were used for the comparison of the data. Pearson and Spearman analyses were used for correlation of the parameters.

Results: MPO levels were found to be high in Nephrotic syndrome cases when compared to healthy controls. Urea, creatinine, BUN, BUN/Cr ratio and eGFR were high in Nephrotic syndrome cases while total protein, albumin, globulin and A/G ratio showed decreased levels. MPO had a positive correlation with creatinine and urine protein-creatinine ratio in Nephrotic syndrome. During remission, MPO levels decreased significantly while total protein and albumin levels increased.

Conclusion: Myeloperoxidase enzyme is found to be elevated and it strongly correlated with the severity of disease in Nephrotic syndrome. Further studies can be done to use MPO as a therapeutic target in Nephrotic syndrome to ameliorate the symptoms.

Keywords: Nephrotic Syndrome, Protein-creatinine ratio, Remission

INTRODUCTION

Neutrophils harvest the intense green iron containing enzyme, Myeloperoxidase (MPO). MPO synthesis begins at the promyelocyte stage of neutrophil development and ends at the myeloid stage, during which the MPO containing azurophilic granules gets distributed to the daughter cells [1]. It is a highly cationic and glycosylated protein having a molecular weight of 144 kD [2]. They are released upon the activation of neutrophils, mostly during a state of inflammation. The peroxidase activity of MPO in the presence of H_2O_2 and chloride as co-substrates forms a major defense against myriad of bacteria, thus making it the key cellular defense mechanism in innate immunity [3].

Paradoxically, accumulating evidences implicated the role of MPO in initiation and progression of atherosclerosis [4]. MPO- H_2O_2-chloride triad results in the generation of many toxic end products like hypochlorite (HOCl), chloramines, hydroxyl radical, singlet oxygen and ozone. These agents when released out of the cell may damage the normal tissues. MPO has been found to be involved even in renal disease [5]. There are studies pointing to the presence of MPO and HOCl modified proteins in diseased renal tissues [6]. MPO is also linked to inflammation and oxidative stress due to its location in leucocytes and the role in catalyzing the formation of oxidative agents.

Nephrotic syndrome is a glomerular disease which causes intense proteinuria. Due to the tissue damage caused by numerous mechanisms, the porosity of the glomerular basement membrane is increased resulting in the leakage of protein eventually leading to an array of clinical symptoms. Role of MPO in the pathogenesis of Nephrotic syndrome has been a less researched subject. But there are substantial evidences available for neutrophils mediating glomerular injury in the nephritis models of rats [7]. Begenik et al., reported an increased MPO activity in adult Nephrotic syndrome patients [8]. Also there are studies which prove that MPO when bound to glomerular basement membrane can react with infused H_2O_2 in a chloride containing medium to induce glomerular injury [9]. Present study deals with the role of MPO and its association with other renal parameters in Nephrotic syndrome thus trying to elucidate a role of MPO in the prognosis of Nephrotic Syndrome.

MATERIALS AND METHODS

The present study comprised of two phases. The first phase was a Case-Control study in which 40 newly diagnosed primary Nephrotic syndrome patients and 40 age and sex matched healthy subjects in the age group of 5-50 years were included. Control subjects were free from any clinical illness. Informed consent was obtained from all the subjects and the study was approved by the Institutional Ethics Committee. Fasting blood samples were collected in a sterile plain vacutainer from which the serum was separated and stored at -80^{0}C until analysis.

Myeloperoxidase was estimated using the method of Matheson et al., [10]. 100 µL serum was added to a mixture of 0.5 ml phosphate buffer (pH–6.1), 0.5 ml H_2O_2 and 0.5 ml 4-aminoantipyrine (substrate). Absorbance was recorded at one min intervals for 5 minutes at 512 nm in a UV-spectrophotometer. Average change in absorbance for one min was calculated. MPO activity was calculated using extinction coefficient of 13,900 $M^{-1}cm^{-1}$.

Serum urea, creatinine, total protein and albumin were measured in semi autoanalyser using commercially available kits. Blood Urea

Nitrogen (BUN), BUN-Creatinine ratio (BUN/Cr), globulin and Albumin-Globulin ratio (A/G) were calculated using the standard formulae. Estimated Glomerular Filtration Rate (eGFR) was calculated using Modified Diet in Renal Disease (MDRD) formula [11]. Protein and creatinine was estimated in the 24 hour urine sample of Nephrotic syndrome patients using turbidimetric methods. Protein creatinine ratio was derived mathematically.

All the Nephrotic syndrome patients were treated with the recommended regime of steroids depending on the type of primary Nephrotic syndrome, following the KDIGO guidelines [12]. They were followed up during their regular check up in the hospital, for a period of ten months, in the second phase of the study. Out of 40 Nephrotic syndrome patients only 15 attained remission in this time period and they were included in the remission group. All the serum and urine analysis were repeated in these subjects.

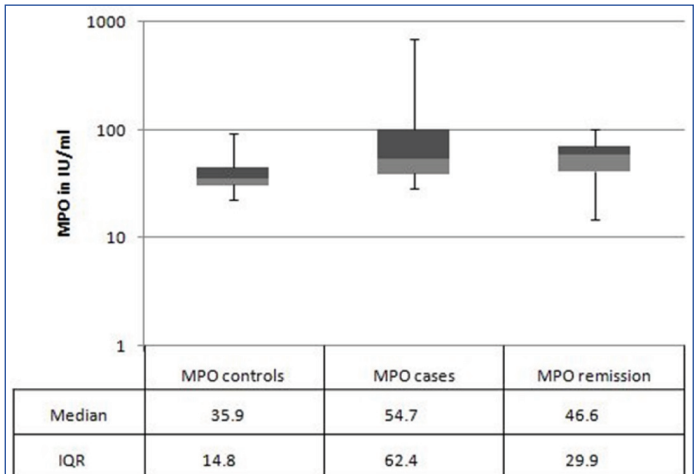
STATISTICAL ANALYSIS

Sample size was calculated based on the statistical formula for paired sample analysis and it was found that 40 cases and 40 controls were adequate for the study. Sample size in remission group was taken according to the number of Nephrotic syndrome patients who attained remission in a time period of ten months. IBM SPSS, version 20.0 was used for the analysis. Quantitative data was expressed as mean ± standard deviation for normally distributed parameters and as median ± Inter Quartile Range (IQR) for non-normal distributions. All biochemical parameters which were normally distributed were analysed using paired students t-test, while those parameters which did not show normal distribution were subjected to Wilcoxon signed rank test. Pearsons and Spearmans correlation analysis was used for the parameters which follow normal and non-normal distribution respectively. $p< 0.05$ was considered statistically significant.

RESULTS

[Table/Fig-1] shows the box plot of MPO levels in Nephrotic syndrome cases, controls and remission group. There was a significant increase in MPO levels in Nephrotic syndrome cases when compared with healthy controls. [Table/Fig- 2] represents the values of renal function parameters in 40 cases and 40 control subjects. Urea, creatinine, BUN, BUN/Cr and eGFR values were higher in Nephrotic syndrome cases while total protein, albumin, globulin and A/G were less in them, when compared to the healthy controls.

[Table/Fig-3] represents the comparison of MPO, renal function parameters and urine protein-creatinine ratio in 15 patients who attained remission, before and after steroid therapy. There was a significant decrease in MPO levels in the remission group when compared to their corresponding values before steroid therapy, while total protein, albumin and A/G ratio showed a significant increase [Table/Fig-3]. Correlation study of MPO showed a significant positive correlation with serum creatinine [Table/Fig-4] and urine protein-



	MPO controls	MPO cases	MPO remission
Median	35.9	54.7	46.6
IQR	14.8	62.4	29.9

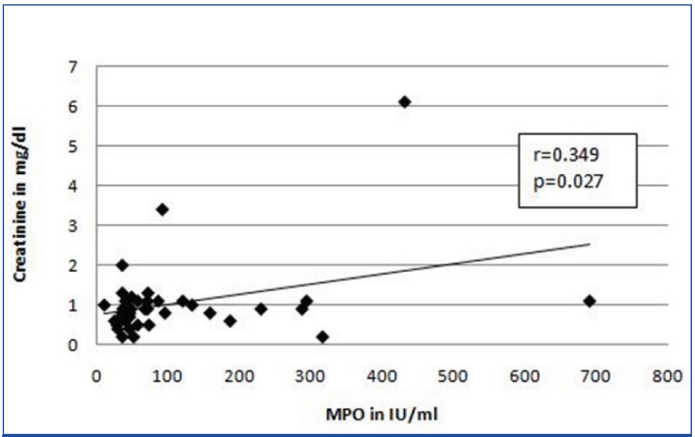
[Table/Fig-1]: Box plot showing MPO levels in healthy controls, cases and remission groups

Sl No	Parameters	Cases (n=40)	Controls (n=40)	p-value
1	Urea	38.52±26.26	22.70±6.51	0.001
2	BUN	18±12.27	10.60±3.04	0.001
3	Creatinine	1.63±0.98	0.86±0.19	0.025
4	BUN/Creatinine	22.79±18.48	12.81±4.40	0.001
5	Total Protein	4.24±1.07	7.69±0.79	<0.001
6	Albumin	1.99±0.88	4.39±0.610	<0.001
7	Globulin	2.25±0.45	3.28±1.08	<0.001
8	A/G	0.91±0.42	1.54±0.71	<0.001
9	eGFR	121.61±102.43	101.37±30.54	0.162

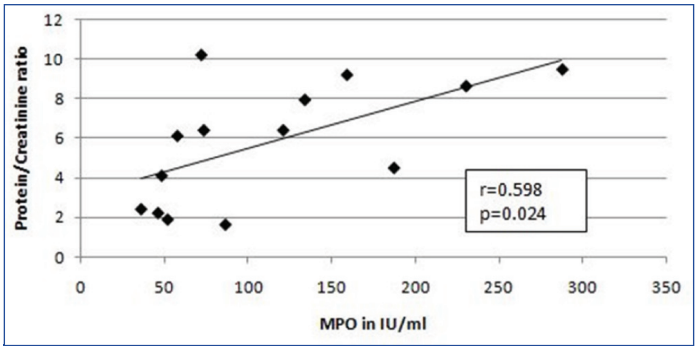
[Table/Fig-2]: Renal function parameters in Nephrotic syndrome cases and controls

Sl No	Parameters	Case (n=15)	Remission (n=15)	p-value
1	Myeloperoxidase	91.5±45.3	44.5±21.6	0.003
2	Urea	42.92±29.17	30.07±8.88	0.057
3	BUN	20.06±13.63	14.05±4.15	0.056
4	Creatinine	1.11±0.67	0.77±0.18	0.078
5	BUN/Creatinine	20.1±12.43	18.54±5.05	0.659
6	Total Protein	4.29±1.10	6.78±1.41	0.001
7	Albumin	2.19±1.10	4.19±0.80	<0.001
8	Globulin	2.10±0.42	2.59±0.91	0.097
9	A/G	1.11±0.62	1.89±1.15	0.046
10	eGFR	94.33±46.38	123.67±40.42	0.069
11	Protein/Creatinine (urine)	5.81±3.06	2.71±1.36	0.045

[Table/Fig-3]: Myeloperoxidase and renal function parameters in Nephrotic syndrome cases before and after steroid therapy



[Table/Fig-4]: Correlation between MPO and Creatinine in Nephrotic syndrome cases



[Table/Fig-5]: Correlation between Protein/Creatinine and MPO in Nephrotic syndrome cases

creatinine ratio [Table/Fig-5]. MPO also showed a weak positive correlation with serum urea and BUN. There was no correlation for MPO with eGFR and protein fractions.

DISCUSSION

Myeloperoxidase is a strongly cationic glycosylated protein with a molecular weight of 144 kD, consisting of two identical dimers linked by a disulfide bridge [13]. Even though it is important in enhancing the immune defense system, individuals possessing a promoter polymorphism and associated 2 fold reduction in MPO expression, was found to be cardio protected [14]. Generation of Reactive Oxygen Species via MPO catalyzed pathways may have a substantial impact on the promotion of inflammatory events. In this study, the MPO levels showed a marked increase in Nephrotic syndrome patients when compared to healthy controls. The primary reason for the increased MPO in Nephrotic syndrome may be as illustrated in [Table/Fig-6] [15,16].

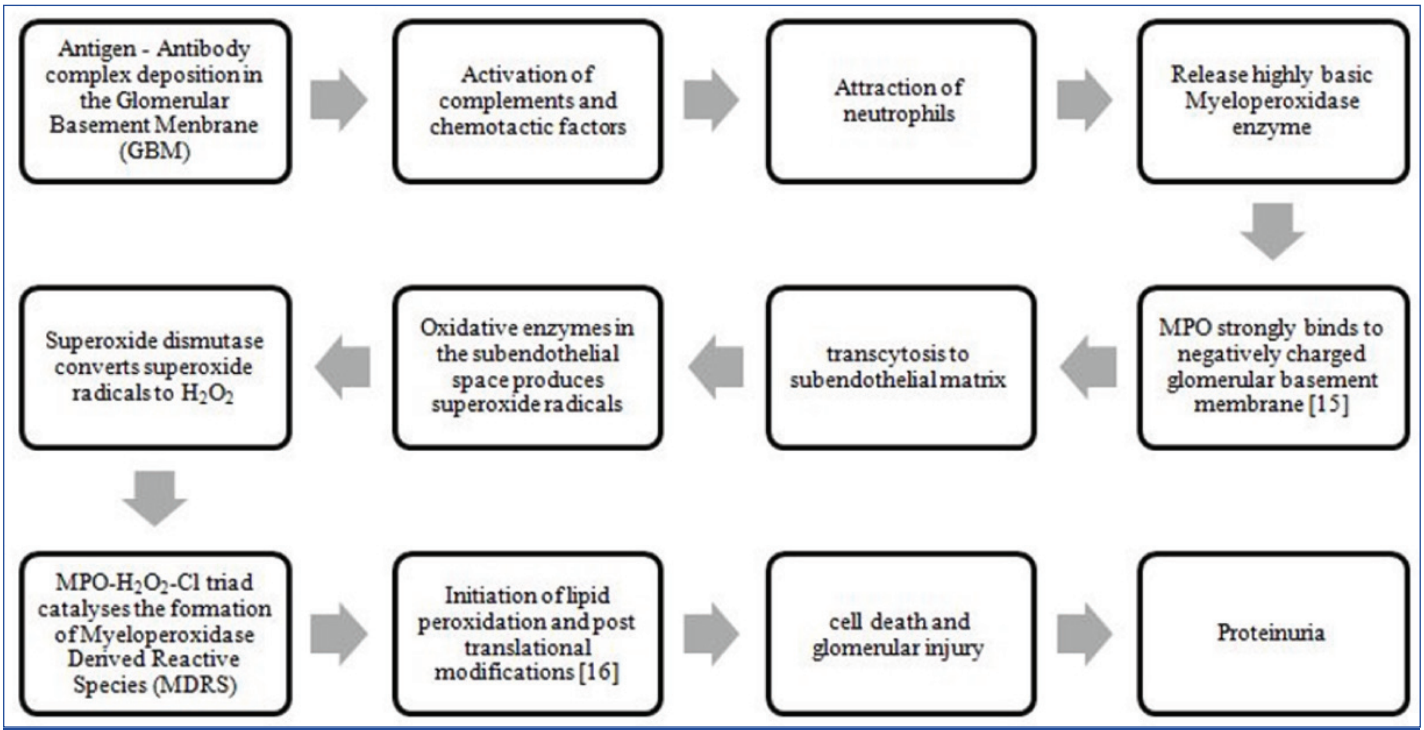
Vaso relaxant Nitric oxide (NO) is also a substrate for MPO and thus MPO acts as a catalytic sink for NO thereby reducing its bioavailability. This may lead to endothelial dysfunction [17]. MPO is known to mediate protein carbamylation which leads to post translational protein modification. This makes the resulting protein unsuitable for enzymatic digestion and results in peptides with unexpected retention time and masses in Mass Spectrometric experiments. Usually uremia secondary to chronic renal disease creates an optimum environment to initiate protein carbamylation, which has been hypothesized as a mechanism that causes repeated infections and constitutes one of the major causes for morbidity and mortality [18]. In this study, the values of urea, creatinine, BUN and BUN/Cr ratio increased in Nephrotic syndrome cases when compared to healthy controls. MPO levels showed a significant positive correlation with creatinine in Nephrotic syndrome cases. Even though not significant, there was a positive correlation for MPO with urea and BUN (r=0.246, p=0.126). Therefore both uremia and increased MPO levels may cause protein carbamylation of circulating protein, mainly the extracellular matrix (ECM) proteins of the glomerulus. Carbamylated ECM proteins induce adhesion of monocytes, which causes subsequent inflammation and tissue damage finally leading to proteinuria [18]. In line with this, the present result points to the presence of hypo albuminemia, hypo proteinemia and hypo globulinemia in Nephrotic syndrome.

MPO and HOCl modified proteins co-localize into the glomerular basement membranes and tubular epithelia which suggest that MPO is a main pathogenic factor in glomerular and tubulointerstitial diseases [5]. When MPO was perfused into the renal arteries of the rats it bound to glomerular capillary wall due to its cationic charge and lead to subsequent platelet influx and degranulation. Thus MPO mediated glomerular disease results in endothelial and mesangial cell injury and subsequent proliferative response. These morphological changes resemble those seen in several forms of inflammatory and proliferatory responses in man [19]. In this study MPO levels showed a significant positive correlation with urine protein-creatinine ratio which is continuously being used in clinical settings for the diagnosis and to assess the severity of the disease. Thus, MPO levels strongly associates with the severity of the disease in Nephrotic syndrome.

On the contrary, in a study conducted in Saudi, MPO levels were found significantly low in chronic renal failure patients. This might be due to the inhibition of uremic toxins [20]. In another study conducted on patients with end stage renal disease it was observed that process of haemodialysis itself resulted in a significant rise in plasma MPO levels [21].

Results obtained during remission showed that there was a significant decrease in MPO levels when compared to the disease state. The levels of urea, creatinine, BUN and BUN/Cr ratio came down, even though not very significant, during remission in Nephrotic syndrome. This might be due to the effect of steroids which reduces the inflammation and thus healing the glomerular basement membrane. Glucocorticoids inhibit the soluble mediators released by the T lymphocytes and also have a direct action on the glomerular podocytes [22]. In glomerulonephritis mouse model, steroid treatment significantly reduced the number of MPO specific plasma cells in the spleen [23].

Thus the components of MPO pathway can be used as attractive targets for the development of prognostic biomarkers and also can be implemented as an important therapeutic target for Nephrotic syndrome. MPO inhibition can be done at different levels either by active site blockage or by diversion from chlorination cycle. Oxidized inhibitors and hypochlorite scavengers can also be used as important therapeutic strategies [24].



[Table/Fig-6]: Schematic representation illustrating the possible cause for increased Myeloperoxidase level in Nephrotic syndrome

CONCLUSION

Myeloperoxidase enzyme is found to be elevated and it strongly correlates with the severity of disease in Nephrotic syndrome and thus can be used as a prognostic marker. Further studies can be done to use MPO as therapeutic target in Nephrotic syndrome to ameliorate the symptoms.

ACKNOWLEDGEMENT

We acknowledge Centre for Scientific and Industrial Research (CSIR), India, for providing the research fellowship to carry out the work.

REFERENCES

[1] Klebanoff SJ. Myeloperoxidase: friend and foe. *J Leukoc Biol*. 2005;77(5):598-625.
[2] Arnhold J. Properties, functions, and secretion of human myeloperoxidase. *Biochemistry (Mosc)*. 2004;69(1):4-9.
[3] Klebanoff SJ, Kettle AJ, Rosen H, Winterbourn CC, Nauseef WM. Myeloperoxidase:a front-line defender against phagocytosed microorganisms. *J Leukoc Biol*. 2013;93(2):185-98.
[4] Shao B, Oda MN, Oram JF, Heinecke JW. Myeloperoxidase: an oxidative pathway for generating dysfunctional high-density lipoprotein. *Chem Res Toxicol*. 2010; 23(3):447-54.
[5] Malle E, Buch T, Grone HJ. Myeloperoxidase in kidney disease. *Kidney Int*. 2003;64(6):1956-67.
[6] Gröne HJ, Gröne EF, Malle E. Immunohistochemical detection of hypochlorite-modified proteins in glomeruli of human membranous glomerulonephritis. *Lab Invest*. 2002;82(1):5-14.
[7] Johnson RJ, Lovett D, Lehourer RI, Couser WG, Klebanoff SJ. Role of oxidants and proteases in glomerular injury. *Kidney Int*. 1994;45(2):352-9.
[8] Begenik H, Soyoral YU, Erkoc R, Emre H, Taskın A, Tasdemir M, et al. Serum malondialdehyde levels, myeloperoxidase and catalase activities in patients with Nephrotic syndrome. *Redox Rep*. 2013;18(3):107-12.
[9] Johnson RJ, Couser WG, Chi EY, Adler S, Klebanoff SJ. New mechanism for glomerular injury. Myeloperoxidase-hydrogen peroxide-halide system. *J Clin Invest.* 1987;79(5):1379-87.
[10] Matheson NR, Wong PS, Travis J. Isolation and properties of human neutrophil myeloperoxidase. *Biochemistry*. 1981;20:325-30.
[11] Kuo CF, Yu KH, Shen YM, See LC. The Chinese Version of the Modification of Diet in Renal Disease Equation is a Superior Screening Tool for Chronic Kidney Disease among Middle-aged Taiwanese than the Original MDRD and Cockcroft-Gault Equations. *Biomed J.* 2014;37(6):398-405.
[12] KDIGO Clinical Practice Guideline for Glomerulonephritis. *Kidney International Supplements*. 2012;2:132-274.
[13] Nauseef WM, Malech HL. Analysis of the peptide subunits of human neutrophil myeloperoxidase. *Blood*. 1986;67(5):1504-07.
[14] Reynolds WF, Stegeman CA, Tervaert JW. -463 G/A myeloperoxidase promoter polymorphism is associated with clinical manifestations and the course of disease in MPO-ANCA-associated vasculitis. *Clin Immunol*. 2002;103(2):154-60.
[15] Johnson RJ, Lovett D, Lehourer RI, Couser WG, Klebanoff SJ. Role of oxidants and proteases in glomerular injury. *Kidney Int*. 1994;45(2):352-59.
[16] Schindhelm RK, van der Zwan LP, Teerlink T, Scheffer PG. Myeloperoxidase: a useful biomarker for cardiovascular disease risk stratification? *Clin Chem*. 2009;55(8):1462-70.
[17] Abu-Soud HM, Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidases. *J Biol Chem*. 2000;275(48):37524-32.
[18] Sirpal S. Myeloperoxidase-mediated lipoprotein carbamylation as a mechanistic pathway for atherosclerotic vascular disease. *Clin Sci (Lond).* 2009;116(9):681-95.
[19] Johnson RJ, Guggenheim SJ, Klebanoff SJ, Ochi RF, Wass A, Baker P, et al. Morphological correlates of glomerular oxidant injury induced by the myeloperoxidase- hydrogen peroxide – halide system of the neutrophil. *Lab Invest*. 1988;58(3):294-301.
[20] Ahmed AM, Elhassan KEH, Abdallah HY, Elabid EH. Evaluation of myeloperoxidase in Saudi patients with Chronic renal failure. *IJHSR.* 2013;3(12):69-74.
[21] Rao AM, Anand U, Anand CV. Myeloperoxidase in Chronic kidney disease. *Ind J Clin Biochem*. 2011;26:28-31.
[22] Taha Y, Raab Y, Carlson M, Larsson A, Lördal M, Lööf L, et al. Steroids reduce local inflammatory mediator secretion and mucosal permeability in collagenous colitis patients. *World J Gastroenterol.* 2006;12(43):7012-18.
[23] Bontscho J, Schoureiber A, Manz RA, Schneider W, Luft FC, Kettritz R. Myeloperoxidase-specific plasma cell depletion by bortezomib protects from anti-neutrophil cytoplasmic autoantibodies-induced glomerulonephritis. *J Am Soc Nephourol*. 2011;22(2):336-48.
[24] Malle E, Furtmüller PG, Sattler W, Obinger C. Myeloperoxidase: a target for new drug development? *Br J Pharmacol*. 2007;152(6):838-54.

PARTICULARS OF CONTRIBUTORS:
1. Senior Research Fellow, Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal University, India.
2. Professor, Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal University, India.
3. Professor, Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal University, India.
4. Associate Professor, Department of Nephrology, Kasturba Medical College, Mangalore, Manipal University, India.
5. Professor and Head, Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal University, India.
6. Associate Professor, Department of Nephrology, Kasturba Medical College, Mangalore, Manipal University, India.
7. Post Graduate, Department of Nephrology, Kasturba Medical College, Mangalore, Manipal University, India.
8. Post Graduate, Department of Nephrology, Kasturba Medical College, Mangalore, Manipal University, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Benedicta D'Souza,
Professor, Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal University-575004, India.
E-mail: benedicta_7@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Dec 15, 2014**
Date of Peer Review: **Apr 06, 2015**
Date of Acceptance: **Apr 09, 2015**
Date of Publishing: **Jun 01, 2015**