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Myeloperoxidase: indicator of cardiovascular disease in chronic kidney disease patients of tertiary care hospital of Karachi

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Abstract

Objective: To estimate the levels of myeloperoxidase in various stages of chronic kidney disease, and to correlate them with an inflammatory marker and lipid profile.

Method: The cross-sectional study was conducted at the Biochemistry Department, Basic Medical Sciences Institute, in collaboration with the Nephrology Department, Jinnah Post Graduate Medical Centre, Karachi, from January 2013 to September 2014, and comprised chronic kidney disease patients and healthy controls. Serum cholesterol, triglycerides, high-density lipoprotein, C-reactive protein and myeloperoxidase levels were noted. Data was subjected to statistical analysis.

Results: Of the 150 subjects, 84(56%) were cases and 66(44%) were controls. Weight, body mass index, triglycerides, very low-density lipoprotein, C-reactive protein and myeloperoxidase levels were significantly higher among the cases compared to the controls ($p<0.05$). Serum myeloperoxidase had a significantly positive association with C-reactive protein ($p<0.01$), cholesterol ($p<0.01$),

triglycerides ($p<0.01$), low-density lipoprotein ($p<0.01$) and very low-density lipoprotein ($p<0.01$), and had a negative correlation with high-density lipoprotein ($p<0.01$).

Conclusion: Myeloperoxidase concentration had association with lipid profile and C-reactive protein.

Key Words: Myeloperoxidase, Cardiovascular, Chronic kidney disease, Lipid profile.

Introduction

One of the biggest worldwide health problems is chronic kidney disease (CKD), a disease associated with considerable morbidity and mortality. The prevalence of CKD is 13.4% worldwide. (1) In southeast Asia, 3% population is facing death due to CKD (2). Pakistan is reported to have an overall CKD prevalence of about 12.5%, and around 21 million are in CKD stages 3 and 4. (3)

Development and progression of CKD is dependent on prime factors like age, dyslipidemia, obesity, smoking, diabetes mellitus (DM) and hypertension (HTN) (4). Diagnosed CKD patients are 10-30 times more at risk of having cardiovascular complications than those with normal kidney functions. (5) The risk of cardiovascular disease (CVD) is increased very early with the progression of CKD at a glomerular filtration rate (GFR) of about 75ml/min, and increases along with decreasing renal function. (6) A non-traditional risk factor is inflammation, which is believed to be a key player in mediating CVDs in CKD patients. The existence of high degree of inflammation in CKD and CVD patients is established by the increased levels of serum C-reactive protein (CRP). (7)

Myeloperoxidase (MPO) is a heme protein, which destabilises the oxidative environment by generating reactive oxidant and diffusible radical species. This initiate lipid peroxidation and promote sequential post-translational modifications of target proteins. (8) Normal tissues can be damaged by MPO-generated oxidants, and this contributes to cellular injury due to inflammation,

thus becoming a potential participant in the progression of heart disease. (9) There are links reported by a study that increase MPO levels and cause heart attacks, and have a probable role in CVD risk even in the healthy population.(10) Patients with elevated risk for imminent cardiac events are identified by their MPO levels, which highlight the potential role of MPO assessment in the starting of CVD events and their risk. (11)

There is very limited data available on the role of MPO in CKD patients. The current study was planned to estimate and compare MPO levels in non-CKD patients against various categories of CKD, and to correlate the levels of MPO with inflammatory marker CRP and lipid profile parameters.

Patients and Methods

The cross-sectional study was conducted at the Biochemistry Department, Basic Medical Sciences Institute (BMSI), in collaboration with the Nephrology Department, Jinnah Post Graduate Medical Centre (JPMC), Karachi, from January 2013 to September 2014. After approval from the JPMC ethics review board, the sample size was calculated with assumed CKD prevalence of 12.5%(12), confidence limit 5.3% using the formula: n = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1)+p*(1-p)](https://www.openepi.com/PDFDocs/SSPropor Doc.pdf) (13) The sample was raised using convenience random sampling from among patients aged 35-75 years at the Nephrology Department without any known CVD. Those excluded were patients of liver disease, acute or chronic inflammatory disease and those on steroid therapy. Healthy controls were recruited from the BMSI. Written informed consent was taken from all the subjects. Those having GFR >90ml/min/1.73m^2 were in control Group A, while CKD patients with GFR <90ml/min/1.73m^2 formed Group B. At the time of enrolment, all the subjects were asked in detail regarding their past medical, surgical and treatment history using a questionnaire. All the participants were requested to come with 10-12 hours overnight fasting for sample collection. The

analysis of biochemical parameters, including lipid profile, was done using spectrophotometry (Merck kits: Cat. No. CH 10085,TG A130016, HDL18109). Friedewald's formula was used to calculate low-density lipoprotein cholesterol (LDL-c)(14). Triglycerides (TGs) were determined by using glycerol-3-phosphate oxidase phenol aminophenanzone (GPO-PAP) method (Merck, France). The estimation of cholesterol was done with enzymatic colorimetric cholesterol oxidase- phenol 4-aminoantipyrine peroxidase (CHOD-PAP) method (Merck, France). Enzyme-linked immunosorbent assay (ELISA) kit was used to estimate serum CRP (Cat. No. KAPDB 4360, DIA source Immuno Assay S.A., Belgium) and MPO (Cat No. ab119605, Abcam, EU, and ROW, UK).

Cockcroft and Gault equation was used to calculate GFR. (15)

Data was subjected to statistical analysis using Mann-Whitney U test to compare means within groups. Data was expressed as mean ± standard deviation. $P<0.05$ was considered significant.

Results

Of the 150 subjects, 84(56%) were cases and 66(44%) were controls. Demographic and biochemical characteristics between the two groups showed significant differences on various parameters (Table 1). When the levels of CRP and MPO were observed in the 5 CKD stages, they showed a increasing trend with decreasing GFR (Table 2).

Serum MPO had significantly positive correlation with CRP, cholesterol, TG, LDL-c and very low-density lipoprotein cholesterol (VLDL-c), and a significant negative correlation with high-density lipoprotein cholesterol (HDL-c) ($p<0.05$) (Figure).

Discussion

The prevalence of CKD is unexpectedly high in Pakistani population due to increased incidence of high blood pressure (BP) and DM (16). The major

complication in these patients is heart disease, and accelerated atherosclerosis has been observed. These problems are more reported in end-stage renal disease (ESRD), and some CVD risk factors are frequent and appear early in CKD (16). The current study compared serum MPO level in different stages of CKD patients and explored the relationship between MPO concentration with lipid and renal profiles as well as with high-sensitivity CRP (hs-CRP).

Results revealed that CKD patients had significant high levels of serum MPO compared to the controls, which is similar to earlier findings (17). Increased level of serum MPO generates numerous oxygen species (OS), and these oxidants play a key role in the formation of atherosclerotic plaque and can promote cardiovascular complications (18). Accumulation of nitrogenous waste products and advanced level of oxidation of lipid and protein not only reduce GFR, but also contribute to the enhanced cardiovascular risk associated with CKD(19). Serum MPO levels were significantly high in CKD stage 3 patients and gradually increased with the decrease in GFR in the current study, indicating that this enzyme has a probable role in the aetiology of cardiovascular complications in CKD patients, which is in contrast to an earlier finding (20).

In the current study, patients with decreased GFR, demonstrates a significant role of MPO in oxidative stress (OS)-mediated endothelial dysfunction by the production of advanced oxidation protein products (AOPP) and advanced glycation end products (AGE), and, consequently, a strong negative correlation between MPO and GFR, which is in contrast to the findings of an earlier study(21).

In the present study, mean BMI of CKD patients was significantly increased compared to the controls. This finding is in agreement with one study (22), with another study (23) reporting that high BMI is linked with aggravated cardiovascular complications in early CKD stages.

The current result showed significant increase of lipid profile in patients compared to controls, and a strong positive correlation of cholesterol, TGs and

LDL-c existed with MPO, while serum HDL-v level was significantly lower of in patients compared to controls and showed significant negative correlation with MPO. These results are in line with literature. (24)

The process of initiation and progression of atherosclerosis is related to increased inflammation in the body. In the present study, serum hs-CRP was used to estimate the inflammation status in the subjects. Results showed patients had higher CRP levels than controls. Other studies (25,26) also observed increased levels of serum CRP in CKD patients. A study (27) reported that in cardiac patients, MPO levels are much higher than in controls. Another study showed that MPO is more predictive of cardiac events than serum CRP. (9)

In the current study, serum MPO levels were considerably high in CKD stage 3, and gradually increased with the decrease in GFR, indicating that the enzyme has a probable role in the pathogenesis of cardiovascular complications in patients diagnosed with CKD, and MPO may be an early predictor of CVD in these patients. Further longitudinal studies are required for the confirmation of the current study's findings.

Limitations of the current study include delayed reporting of results. Also, CKD patients could have been classified into stages using the (National kidney foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines (28), and other cardiovascular risk factors, such as homocysteinaemia, high-sensitivity lipoprotein and OS, and ultrasonographic measurements, such as the measurement of increased intima-media thickness (IMT) of large arteries, were not involved.

Conclusion

There was a significant increase in serum MPO concentration in CKD patients with the progression of the disease.

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References

1. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global prevalence of chronic kidney disease–a systematic review and meta-analysis. PloS one 2016; 11(7):e0158765. Doi: doi: 10.1371/journal.pone.0158765.
2. Misra A, Tandon N, Ebrahim S, Sattar N, Alam D, Shrivastava U, et al. Diabetes, cardiovascular disease, and chronic kidney disease in South Asia: current status and future directions. bmj. 2017;357:j1420. doi: https://doi.org/10.1136/bmj.j1420
3. Jessani S, Bux R, Jafar TH. Prevalence, determinants, and management of chronic kidney disease in Karachi, Pakistan-a community based cross-sectional study. BMC Nephrol. 2014;15(1):90. doi: 10.1186/1471-2369-15-90.
4. Kazancioğlu R. Risk factors for chronic kidney disease: an update. Kidney Int Suppl. 2013;3(4):368-71.
5. Kochi M, Kohagura K, Shiohira Y, Iseki K, Ohya Y. Chronic kidney disease, inflammation, and cardiovascular disease risk in rheumatoid arthritis. Journal of cardiology. 2018 Mar 1;71(3):277-83.
6. Alani H, Tamimi A, Tamimi N. Cardiovascular co-morbidity in chronic kidney disease: Current knowledge and future research needs. *World J Nephrol*. 2014;3(4):156–168. doi:10.5527/wjn.v3.i4.156

7. Quiroga B, Verdalles Ú, Reque J, Garcia de Vinuesa S, Goicoechea M, Luño J. Cardiovascular events and mortality in chronic kidney disease (stages I-IV). Nefrología (English Edition). 2013 Jul 1;33(4):539-45.
8. Mark PB. Strategies to manage cardiovascular risk in chronic kidney disease. Nephrology Dialysis Transplantation. 2018 Jan 1;33(1):23-5.
9. Agarwal A, Hegde A, Yadav C, Ahmad A, Manjrekar PA, Srikantiah RM. Assessment of oxidative stress and inflammation in prediabetes—A hospital based cross-sectional study. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2016 Apr 1;10(2):S123-6.
10. Hazen, Stanley L. "Myeloperoxidase and plaque vulnerability." Arteriosclerosis, Thrombosis, and Vascular Biology. 2004;24: 1143–6.
11. Agarwal A, Hegde A, Yadav C, Ahmad A, Manjrekar PA, Srikantiah RM. Association of interleukin-6 and myeloperoxidase with insulin resistance in impaired fasting glucose subjects. Indian Journal of Clinical Biochemistry. 2017 Mar 1;32(1):33-8.
12. Jessani S, Bux R, Jafar TH. Prevalence, determinants, and management of chronic kidney disease in Karachi, Pakistan-a community based cross-sectional study. BMC Nephrol. 2014;15(1):90. doi: 10.1186/1471-2369-15-90.
13. Schaeffer RL, Mendenhall W, Ott L. Elementary Survey Sampling, Fourth Edition. Duxbury Press, Belmont, California 1990.
14. Friedewald, W. T., R. I. Levy and D. S. Fredrickson. "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge." Clinical chemistry. 1972; 18(6): 499-502.
15. Cockcroft DW and Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16(1): 31-41.

16. Imran S, Shiekh A, Saeed Z, Khan SA, Malik AO, Patel J. Burden of chronic kidney disease in an urban city of Pakistan, a cross-sectional study. J Pak Med Assoc. 2015 Apr 1;65(4):366-9.

17. Tsai MS, Shaw HM, Li YJ, Lin MT, Lee WT, and Chan KS. Myeloperoxidase in Chronic Kidney Disease: Role of Visceral Fat. Nephrology 2014; 19(3):136–42. doi: 10.1111/nep.12187.

18. Afshinnia F, Zeng L, Byun J, Gadegbeku CA, Magnone MC, Whatling C, et al. Myeloperoxidase levels and its product 3-chlorotyrosine predict chronic kidney disease severity and associated coronary artery disease. American journal of nephrology. 2017;46(1):73-81.

19. Sun J, Axelsson J, Machowska A, Heimbürger O, Bárány P, Lindholm B, et al. Biomarkers of cardiovascular disease and mortality risk in patients with advanced CKD. Clinical Journal of the American Society of Nephrology. 2016 Jul 7;11(7):1163-72.

20. Madhusudhana RA, Anand U, and Anand CV. Myeloperoxidase in Chronic Kidney Disease. Ind J Clin Biochem. 2011; 26(1):28-31. https://doi.org/10.1007/s12291-010-0075-1

21. Ahmed AM, Elhassan KEH, Abdallah HY, Elabid BEH. Evaluation of myeloperoxidase in Saudi patients with chronic renal failure. Int J Health Sci Res. 2013; 3(12):69-74.

22. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global prevalence of chronic kidney disease–a systematic review and meta-analysis. PloS one. 2016;11(7).

23. Bansal N, Vittinghoff E, Plantinga L, Hsu CY. Does chronic kidney disease modify the association between body mass index and cardiovascular disease risk factors? J Nephrol. 2012; 25(3):317–24. doi: 10.5301/JN.2011.8454.

24. Nichols GA, Philip S, Reynolds K, Granowitz CB, Fazio S. Increased cardiovascular risk in hypertriglyceridemic patients with statin-controlled

LDL cholesterol. The Journal of Clinical Endocrinology & Metabolism. 2018 Aug;103(8):3019-27.

25. Wang J, Tan GJ, Han LN, Bai YY, He M, Liu HB. Novel biomarkers for cardiovascular risk prediction. Journal of geriatric cardiology: JGC. 2017 Feb;14(2):135.

26. Peev V, Nayer A, Contreras G. Dyslipidemia, malnutrition, inflammation, cardiovascular disease and mortality in chronic kidney disease. Current opinion in lipidology. 2014 Feb 1;25(1):54-60.

27. Tejaswi G, Suchitra MM, Rajasekhar D, Kiranmayi VS, Rao PS. Myeloperoxidase, protein carbonyls and oxidative stress in coronary artery disease. Journal of Indian College of Cardiology. 2017 Dec 1;7(4):149-52.

28. JM, Wilson PW, Fried L. KDOQI US commentary on the 2013 KDIGO Clinical Practice Guideline for Lipid Management in CKD. American Journal of Kidney Diseases. 2015 Mar 1;65(3):354-66.

Table 1: Demographic and biochemical characteristics.

Variable	**Group – A** (n= 66) GFR <90 ml/min/1.73m^2	**Group – B** (n = 84) GFR >90ml/min/1.73m^2	**P value**
Age (years)	54.7 ± 5.39	56.58± 5.87	0.187
Weight (Kg)	61.53 ± 8.88	86.45 ± 6.19	<0.001
Height (cm)	162.53 ± 10.01	164.83 ± 7.83	0.465
Body Mass Index (Kg/m^2)	24.85 ± 3.95	28.09 ± 1.25	<0.001
Fasting Blood Sugar (mg/dl)	107 ± 4.35	146.13 ± 26.69*	<0.001
Urea(mg/dl)	51.06 ± 5.63	119.23 ± 34.24	<0.001
Creatinine (mg/dl)	0.74 ± 0.19	1.24 ± 0.14	<0.001
GFR (ml/min/1.73m^2)	108.38 ± 9.78	33.11 ± 7.87	<0.001
Cholesterol (mg/dl)	194.00 ± 41.25	227.38 ± 25.25	0.099
Triglyceride (mg/dl)	121.90 ± 39.28	186.04 ± 31.93	<0.001
HDL-C (mg/dl)	49.83 ± 15.65	31.01 ± 1.23	<0.001
LDL-C (mg/dl)	119.90 ± 42.18	157.72 ± 39.59	0.096
VLDL (mg/dl)	25.27 ± 4.23	37.35 ± 4.31	<0.001
C-Reactive Protein(mg/L)	0.9 ± .23	7.36 ± 2.4	<0.001
Myeloperoxidase(ng/ml)	62. 00 ± 30.26	185.01 ± 39.45	<0.001

GFR: Glomerular filtration rate, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, VLDL: Very low-density lipoprotein-cholesterol.

Table 2. Levels of Serum CRP and MPO in the different stages of CKD.

Variable	Control GFR (>90 ml/min/1.73m^2)	CKD2 GFR (60-89 ml/min/1.73m^2)	CKD3 GFR (30-59 ml/ min/1.73m^2)	CKD4 GFR (15-29 ml/min/1.73 m^2)	CKD5 GFR (<15 ml/min/1.73m^2)
C-Reactive Protein (mg/L)	1.80 ± 1.34	3.92 ± 1.68*	5.74 ± 2.18*□	6.83 ± 2.23*□	9.09 ± 1.76*□ΔΔ
Serum Myeloperoxidase (ng/ml)	43.04 ± 26.36	60.35 ± 18.93	118.47 ± 41.98*□	183.23 ± 37.97*□Δ	249.36 ± 45.77*□ΔΔ

Values are expressed as Mean ± SD,
*Statistically significant as compared to Control p<0.01,
□ Statistically significant as compared to CKD2 p<0.01,
ΔStatistically significant as compared to CKD3 p<0.01,
ΔStatistically significant as compared to CKD4 p<0.01
CKD: Chronic kidney disease, **CRP: C-reactive protein.**

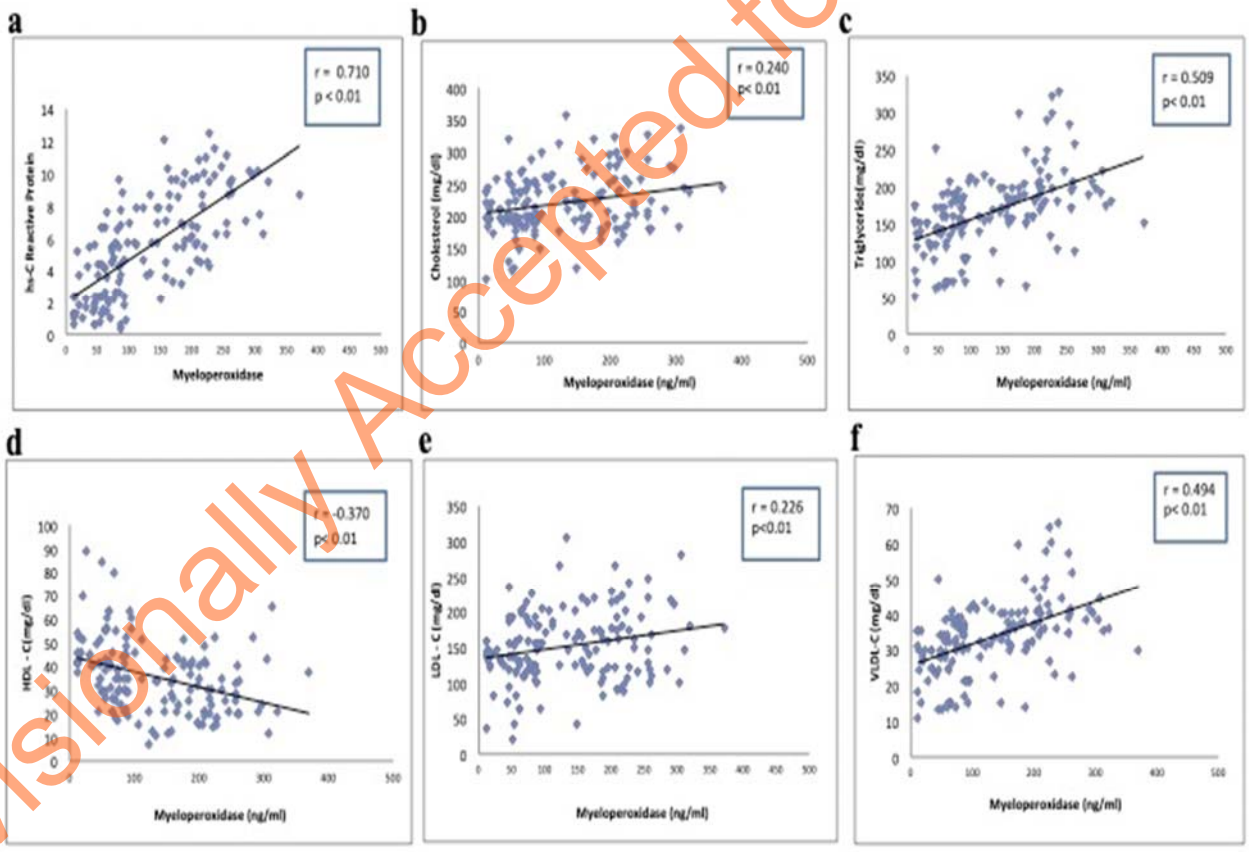

Figure: Graphical representation of myeloperoxidas (MPO) correlation with a) high-sensitivity C-reactive protein (hs-CRP), b) cholesterol, c) triglyceride, d) High-density lipoprotein-cholesterol **(HDL-c), e)** Low-density lipoprotein-cholesterol **(LDL-c) and f) very** low-density lipoprotein-cholesterol **(VLDL-c).**