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STUDY THE ALTERATION OF ANTIOXIDANTS AND MALONDIALDEHYDE IN HEMODIALYSIS PATIENTS WITH CHRONIC RENAL FAILURE IN TAIZ, YEMEN

Dr. Salwa AL-Shamiri*

***Corresponding Author: Dr. Salwa AL-Shamiri**

Assistant Professor in Clinical Biochemistry, Department of Biochemistry, Faculty of Medicine and Health Sciences. Taiz University.

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ABSTRACT

Background: Chronic Renal Failure is occur with exhausted oxidative stress, which is a sequel of increasing in the production of reactive oxygen forms accompanied with the impaired antioxidant defense. **Objectives:** The aim of this study is to detect the alteration in the antioxidants and oxidative stress that influenced by hemodialysis in chronic renal failure patients. **Methods:** Blood samples were drawn from thirty controls and sixty patients with chronic renal failure at the artificial kidney units of Al-Thawra Teaching Hospital in Taiz, Yemen in the period from September 2014 to January 2015. In this study, catalase, glucose-6-phosphate dehydrogenase activities, endogenous non-enzymatic antioxidants, and malondialdehyde were measured in the plasma of patients before and after dialysis. **Results:** Plasma level of malondialdehyde was significantly increased in pre and post hemodialysis groups as compared to the control group and it was found to be lower in post hemodialysis group when compared with pre hemodialysis group. The plasma activity of glucose-6-phosphate dehydrogenase was significantly decreased in pre and post hemodialysis groups as compared with control group. The significant decreasing in catalase activity was presented in the post hemodialysis group as compared with the pre hemodialysis group. **Conclusions:** The significant decreasing in most antioxidative studied with increasing in malondialdehyde level in hemodialysis groups are strongly showed the incidence of oxidative stress and lipid peroxidation that may be accompanied to uremia and the dialysis procedure itself.

KEYWORDS: Malondialdehyde; Oxidative stress; Hemodialysis; Antioxidants; Glucose-6-phosphate dehydrogenase; Catalse.

INTRODUCTION

Chronic renal failure (CRF) is defined as one type of kidney diseases with serious harm characterized by progressive loss of renal function and decreased in glomerular filtration, disruption in extracellular fluid volume, electrolyte, acid-base balance, and retention of nitrogenous waste from protein catabolism.[1] Patients suffering from CRF are exposed to increase oxidative stress that is related to uraemia and the dialysis procedure itself.[2] CRF is a pro-oxidant state, characterized by increasing levels of free radical oxidants relative to antioxidants and increased oxidative stress (OS).[3,4] OS is a state in which reactive oxygen species (ROS) can cause oxidation of cellular and matrix macromolecules including sugars, proteins, deoxyribonucleic acid, bases, and lipids. Increasing evidence suggests that OS may have a role to develop complications in patients with end stage renal disease requiring dialysis.[5] Patients with CRF, including those receiving regular long-term hemodialysis have the high incidence of premature cardiovascular disease.[6] OS plays an important role in the pathogenesis of vascular injury and progression of atherosclerosis and thus relate to endothelial dysfunction and cardiovascular outcomes in patients with chronic kidney disease and patients on maintenance hemodialysis (HD).[6,7] Several reasons have been advanced for the increased OS in patients with CRF and hemodialysis. These include retention of phenol indoxyl carboxyl, methyl guanidine metals, and oxidized lipids, which are oxidants or pro-oxidants and should be excreted in urine or eliminated.[11] Another reason is the continual generation of ROS by the recurrent important and obligatory priming of neutrophil through their interaction with the dialysis membrane.[9] Such interaction and activation of neutrophils have a propose happen due to dialyzer incompatibility.[9,10] In addition to the deterioration in the metabolic activities of the kidneys with decreased various enzymatic and non-enzymatic antioxidative systems.[8,11] Malondialdehyde (MDA) is an intermediate production of polyunsaturated fatty acids. It is widely used as a marker of OS in many researches.[20,23] It has been detected in the plasma and blood cells of hemodialyzed patients.[5,11] Antioxidants are molecules which can interact with free radicals and end the chain reaction before vital molecules damaged.[1] The most important antioxidative enzymes are superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione transferase, and

glucose-6-phosphate dehdrognase.[2,3] Catalase is an enzyme that catalyzing the conversion of hydrogen peroxide to water and oxygen. Although there are several extracellular non enzymatic antioxidants free radical scavengers as carotenoids, vitamin E, and vitamin C. The compounds containing the SH group as glutathione, cysteine, homocysteine, coenzyme Q have antioxidant properties, since the thiol group is a reducing agent and can be reversibly oxidized and reduced. Metal ion binding proteins as ceruloplasmin, transferrin and ferritin and even albumin contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell.[3] Albumin, bilirubin, and urate may scavenge free radicals directly.[12] Cross-sectional studies of chronic renal failure, hemodialysis and their complications are limited in Yemen. The current study is considered to be one of the few studies that have been conducted in Taiz, Yemen; it aims to detect the alteration of oxidative stress and antioxidants states in chronic renal failure patients receiving hemodialysis.

MATERIALS AND METHODS

The present study included CRF patients undergoing hemodialysis in the unit of artificial kidney in Al-Thawra Teaching Hospital, Yemen, Taiz in the period from September 2014 to January 2015. A total of 60 patients (30 men and 30 women, mean age is 26.17 ±0. 57 years) with end stage renal failure who were stayed for hemodialysis were volunteered in this study. All patients had been on regular hemodialysis for at least three months and were dialysis three times weekly each time for 3-5 hours. We excluded from this study all patients suffering from other diseases, such as diabetes, inflammatory diseases, hepatic or respiratory diseases as well as smokers and those on antioxidant vitamins. Thirty apparently healthy patients (15 males and 15 females) with similar age were participated in this study as control group.

Sample collection

Five milliliters of venous blood were drawn from patients before and after hemodialysis as well as from the control group. The aspirated blood samples were divided into two parts, the first whole blood samples were used for glucose 6 phosphate dehydrogenase (G6PD) determination, where the other separated plasma fractions were used for others biochemical determination.

Biochemical analyses

The separated plasma samples were used for the assay of catalase (CAT) activity, malondialdehyde (MDA), albumin (Alb), uric acid (UA), total protein (TP), total biliurobin (TB), and direct biliurobin (DB) levels by using spectrophotometric methods and following the protocols of commercial kits manufacturers from Spinreact, Spain.

Catalase assay: CAT activity was determined in the plasma according to the method of Goth, [13] it was a combination of optimized enzymatic conditions and the spectrophotometric assay of hydrogen peroxide based on the formation of its stable complex with ammonium molybdate. Results were expressed as Katal unit per liter (KU/L).

Malondialdehyde assay: MDA as an index of changes in lipid peroxidation was estimated in the plasma as thiobarbituric acid reactive substances. Results were expressed as (nmol/ml).[14]

Glucose 6 phosphate dehydrogenase assay: The erythrocyte G6PD activity was determined following the oxidation of glucose-6-phosphate to 6-phosphogluconate by conversion of oxidized form of nicotine amide adenine dinucleotide phosphate (NADP) to the reduced form NADPH. The increasing in the absorbance of NADPH at 340 nm was a reflection of G6PD activity. The activity of G6PD is expressed as U/10^{12} RBCs; the ready-made kit of (G6PD*)* assay was purchased from Trinity Biotech, Ireland.[15]

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). The data were analyzed by using student t-test and two-ways analysis of variant (ANOVA) to compare among different groups. P-Value less than 0.05 were considered statistically significant. All analyses were performed using Statistical Package for Social Sciences (SPSS) software package version 20. (SPSS) Inc. Chicago, Illnois, USAT).

RESULTS

The mean plasma MDA levels in both HD groups were significantly higher than control group ($p<0.01$), the post-HD MDA level had decreased from the pre-HD level (Figure 1). The mean activity of CAT was increased in pre-HD group as compared with control group ($p<0.001$). Moreover, the CAT activity of post-HD group was significantly lower than pre-HD group (Figure 2). G6PD were significantly lower in pre-HD and post-HD groups as compared with control group, but the activity of G6PD in post-HD was not significantly different from pre-HD (Figure 3).

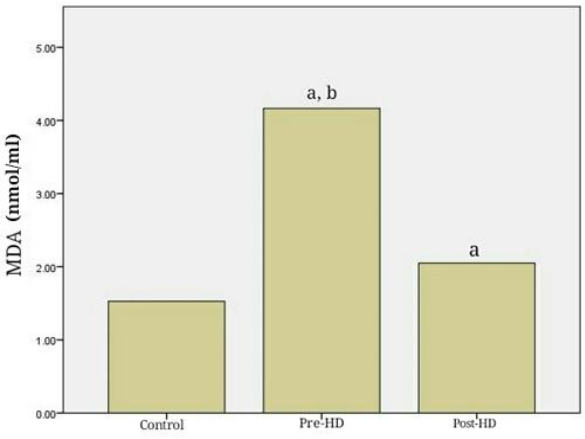

Figure 1: Mean levels of malondialdehyde (nmol/ml) in the plasma of Pre-HD and Post-HD groups of (60) patients with chronic renal failure and (30) controls. [a] (p<0.001) compared with control group, [b](p<0.001) compared with post-HD.

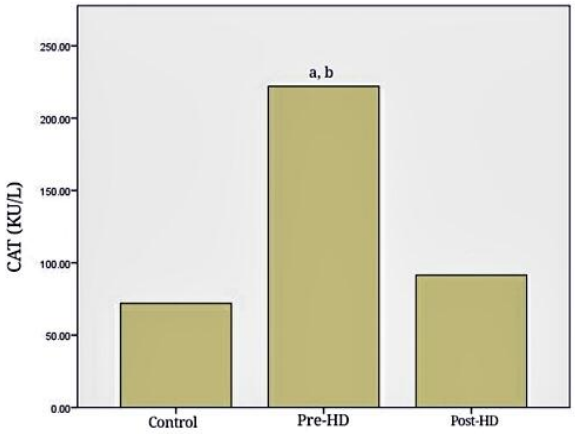

Figure 2: Mean catalase activities (KU/L) in the plasma of Pre-HD and Post-HD groups of (60) patients with chronic renal failure and (30) controls. [a] (p<0.001) compared with control group, [b](p<0.001) compared with post-HD.

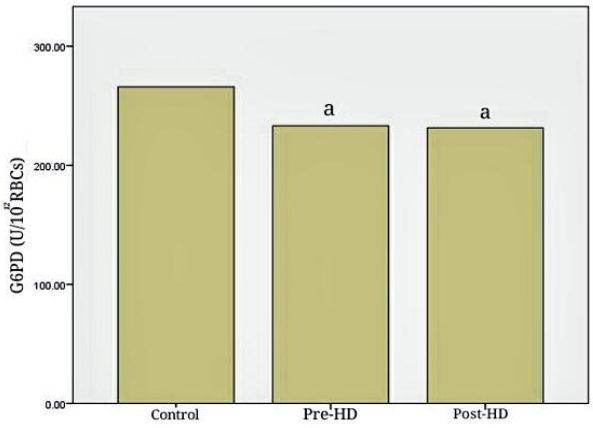

Figure 3: Mean glucose-6-phosphate dehydrogenase activities (U/10[12]RBCs) in Pre-HD and Post-HD groups of (60) patients with chronic renal failure and (30) controls. [a] (p<0.001) compared with control group, [b](p<0.001) compared with post-HD.

The mean plasma levels of Alb and TP were significantly lower in both HD groups than control group. However, the post-HD plasma levels of Alb and TP were significantly higher than pre-HD (Table1).

The mean level of pre-HD UA was significantly higher as compared with control group (p<0.001), whereas the decreasing in UA level of post-HD was not significantly different from control group. In addition, the mean plasma level of UA was significantly decreased in post-HD group as compared to pre-HD group (Table1). The mean plasma levels of TB, DB were significantly lower in both HD groups than control group; moreover, the post-HD levels of both TB and DB were significantly decreased as compared to pre-HD levels (Table 1).

Table1: Mean ± SEM of plasma levels of non-enzymatic antioxidants in Pre-HD and Post-HD groups of (60) patients with chronic renal failure and (30) controls.

Parameters	Control	Pre- HD	Post-HD
Albumin (g/dL)	4.1±0.101	2.7±0.119[a,b]	4.5±0.128[a]
Total protein (g/dL)	6.9±0.112	4.2±0.156[a,b]	6.1±0.182[a]
Uric acid (mg/dL)	3.6±0.097	6.5±0.284[a,b]	3.2±0.155
Total biliurobin (mg/dL)	0.64±0.056	0.31±0.020[a,b]	0.21±0.019[a]
Direct biliurobin (mg/dL)	0.184±0.015	0.180±0.016[a,b]	0.093±0.014[a]

[a] (p<0.001) compared with control group, [b](p<0.001) compared with post-HD.

DISCUSSION

Oxidative damage occurs when the production of ROS exceeds the capacity of the antioxidant defense system.[4] Dialysis procedure can be originated ROS on the surface of dialysis membranes by activation of polymorphnuclear leukocytes.[9] The present study revealed that the MDA levels were significantly increased in Pre-HD group as compared with both control and Post- HD groups. These findings are in agreements with those of Samouilidou and Grapsa,[5] showed that the plasma MDA was increased significantly in Pre- HD and with Ozden *et al*,[16] mentioned in that the plasma level of MDA of hemodialysis patients is increased significantly from that of controls. Plasma MDA is a small water-soluble molecule, and is cleared by the dialysis. Hence, if there is no generation, the levels of MDA are expected to decrease like creatinine at the end of the dialysis session.[7] Prabhakare *et al*,[12] were observed a significant increase in MDA level after correcting for creatinine and they suggested that, the plasma MDA is both generated and cleared during dialysis. These observations may also explain the significant decrease of MDA in post- HD in the present study.

Increasing in CAT activity and glutathione peroxidase occur in response to increase hydrogen peroxide and dismutation superoxide radicals by super oxide dismutase.[12] NADPH is a co-enzyme produced by pentose phosphate pathway. It is involved in generation of glutathione disulfide, which is essential for detoxifying hydrogen peroxide through the reaction catalyzed by the glutathione peroxidase.[3,5] The lower activity of G6PD of the present study may be explained by the inhibitory effect of some uremic toxins such as the guanidine compounds and 3- deoxyglucosone.[17] Furthermore, abnormal red blood cell metabolism seen in uremia may exacerbate G6PD inactivation. The accumulation of certain metabolites like adenosine nucleotides are known in uremia. Substances like ATP are reported as a potent inhibitors of the enzyme G6PD.[18] Although some researchers argue against elevation in the activity of red blood cell enzymes after dialysis since erythrocytes lack the machinery of protein synthesis produced by G6PD.[10] Free radicals generated due to incompatible dialysis membrane might cause consumption of antioxidants[19] that may explain the lower G6PD activity in patients of our study and may be due to increase in MDA level, which can cross link with amino group of protein to form intermolecular cross-links; thereby inactivating several membrane bound enzymes.[20]

Bilirubin, uric acid and plasma albumin concentrations are the primary defense against OS in extracellular fluids, generated during normal metabolism or introduced in the body by consume dietary products rich in antioxidants.[21] Human albumin antioxidant functions include inhibiting copper-stimulated lipid peroxidation reactions and scavenging hypochlorous acid and peroxy radicals. It can also bind free fatty acids and protecting them from peroxidation.[22] Albumin can react with most oxygenated species, which could lead to some oxidation-induced changes.[23] The antioxidant effect of albumin could be resulted from its high content in cysteine residues included in thiolate clusters and various amino acid residues. The thiolate group of the protein contributes physiologically to the redox balance and modulates oxidative stress.[24] The finding of Soejima *et al*,[25] support such a role for albumin, that patient with hypoalbuminemia demonstrate a greater degree of erythrocyte membrane lipid peroxidation than do patients with normal serum albumin concentrations. Inflammation and plasma volume expansion reduced albumin concentration and are independent cardiovascular risk factors in CRF[26] these may explain the decrease of Alb level before dialysis. However, increasing of Alb and TP levels in Post-HD may be revered to hemoconcenteration occurring during the dialysis.[27]

UA is a powerful antioxidant and is a scavenger of single oxygen and other radicals. It is a major contributor to total antioxidant capacity as ferric reducing ability of plasma.[12] Increased synthesis in chronic kidney diseases are connected with produce superoxide anion and hydrogen peroxide, which may play an important role in the pathogenesis of the disease.[2] Decreasing in the post-HD uric acid level of our study may be related to increase lipid peroxidation in hemodialysis patients.[23]

Bilirubin is an endogenous circulating antioxidant, bound to albumin, and retained in the vascular compartment.[28] It is the product of hem catabolism, and is generally regarded as a potentially toxic compound when accumulated at abnormally high concentrations in tissues.[25] Bilirubin may confer the effectiveness of antioxidant properties, giving an important protection against inflammation and atherosclerosis. The antioxidant properties of bilirubin are indicating by its ability to scavenge peroxyl radicals and to attenuate LDL oxidation.[30] Boon *et al*,[28] found that the mildly elevated of bilirubin is associated with protection from kidney damage and dysfunction, in addition to cardiovascular events and all-cause mortality in patients undergoing hemodialysis. Bilirubin protects protein and lipids from oxidation; it provides an important antioxidant capacity pool, by maintaining circulating thiol status. Serum bilirubin may provide useful prognostic information in chronic hemodialysis patients.[29]

CONCLUSION

The results collectively suggest that increasing in MDA level with decreasing in the levels of most antioxidants estimation are more likely due to over consumption of antioxidants by ROS, which producing during hemodialysis and enhancing lipid peroxidation in CRF patients on hemodialysis that could be related to defective kidney functions and the dialysis procedure itself.

RECOMMENDATIONS

This work will be the first step for further researches that might contribute to decrease the complications of oxidative stress and the mortality among patients receive hemodialysis by

- Increasing antioxidants statues by giving different exogenous supplements of vitamins and good nutrition.
- Decreasing lipid peroxidation and oxidative stress by ameliorating the hemodialysis procedures including dialysis membrane and water systems.
- The enzymatic and non-enzymatic antioxidants evaluated in the present study may still contribute to monitoring the patients undergo hemodialysis.

ETHICAL APPROVAL

Approval for this study was obtained from the Ethical Review Committee in the Biochemistry Department, Faculty of Medicine and Health Sciences, Taiz University. All of the participants were informed about the project and provided with written consent.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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