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EVALUATION OF TRACE ELEMENTS AND ANTIOXIDANTS IN PRE AND POST HEMODIALYSIS OF CHRONIC RENAL FAILURE PATIENTS

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ABSTRACT
Oxidative stress is related to several diseases, including chronic renal insufficiency. The lipid peroxidation in hemolysis patients may be partly due to the trace elements disturbances. It has been mentioned that during hemolysis there are relations between deficiency in trace elements and antioxidant levels. The present study was carried out to evaluate the differences between hemodialysis patients and control group according to selenium (Se), aluminium (Al), Zinc (Zn), Copper (Cu), Manganese (Mn), malondialdyhyde (MDA), reduced form glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity levels. The study comprised with 25 hemodialysis patients. The blood samples were taken before and after hemodialysis session. The control group included 11 healthy volunteers. SOD, CAT, (Al) and MDA levels showed significant increase and GSH, Cu, Mn and Zn levels were significant reduced in pre-hemodialysis group when compared with the controls. Moreover, after hemodialysis, the levels of SOD, CAT, (Al) and MDA were significantly elevated and (Cu, Mn and Zn) levels were decreased compared to the control group. On the other hand, no significant changes in the values of GSH and (Se) between control and after dialysis process. From our findings, we concluded that there may be a relation between increased antioxidant enzyme activity levels and MDA and trace elements in chronic renal failure patients. In addition, the levels of aluminium can be used as an important marker in hemodialysis patients.

KEY WORDS: Hemodialysis, antioxidants, oxidants, trace elements, Chronic renal failure.

INTRODUCTION

Chronic renal failure (CRF) is often a complication of sepsis, trauma or multiple organ dysfunctions. The primary event leading to renal failure is a free radical mediated injury to the endothelial cells in the outer medulla. During hemodialysis essential kidney functions, such as the elimination of water and metabolic wastes as well as the correction of the electrolyte and acid/base state are replaced by the artificial purification system (Krachler *et al*., 2000). Samouilidou (2003) reported that hemodialysis has been successful in extending life span of renal patients and is effective in correcting the metabolic abnormalities related to renal oxidative stress that contributes to morbidity in hemodialysis patients. Dialysis patients are subjected to an oxidative stress resulting from the dialysis sessions (Yavuz *et al*., 2004). Oxidative stress occurs when there is an excessive free radical production and/or low antioxidant defense and results in chemical alterations of biomolecules which cause structural and functional modifications (Ozden *et al*., 2004). Free radicals may cause lipid peroxidation and damage macromolecules resulting disturbance in the detoxication enzyme (e.g. SOD) that detoxifies free radicals (Ghoreshi *et al*., 2000). Some of the trace elements, Se, Zn, Mn and Cu are cofactors or structural components of antioxidant enzymes. Moreover, Selenium and glutathione peroxidase (GSH-PX) play an important role in protecting cell membranes from oxidative damage and decreased blood selenium and (GSH-PX) are common in chronic renal failure (Adamowicz *et al*., 2002, Boosalis, 2008). The main purpose of this work was to investigate blood levels of trace elements (Se, Cu, Zn, Mn, Al) and MDA, GSH as well as activities of SOD and CAT in hemodialysis patients with chronic renal failure before and after the dialysis process and compared with control group.

MATERIALS AND METHODS:

Subjects: The present study was conducted on 25 hemodialysis patients of both sexes in the age ranging from 20 to 55 years from Nephrology department Mansoura University Hospital. The control group comprised of 11 healthy volunteers.

Samples Collection: Blood samples were obtained from the concerned patients just before (group 1) and after (group 2) the dialysis process of dialysis and control group in heparinized tubes. Plasma is separated as soon as the blood taken then the erythrocytes were washed with saline solution three times and hemolysed by diluting with deionized water. Hemoglobin (HB) contents of the samples were measured as described by Wintrobe *et al.* (1965). The hemolysate was kept in -70 ^{o}C.

Biochemical Assays

1. **Superoxide dismutase (SOD):** The activity of SOD was assayed by applying the method of Nishikimi *et al.* (1972). The activity of SOD was measured by monitoring the rate of inhibition of NBT reduction.
2. **Catalase**: The activity was determined by the method of Aebi (1984). The principle of the assay is based on the determination of the rate constant of the hydrogen peroxide decomposition by measuring the absorbance changes per minute.

3. Lipid peroxidation: LPO level in plasma was estimated by measuring thiobarbituric acid (TBA) reactive substance (TBARS) as an index of malonialdehyde (MDA) production according to the method of Draper and Hadley (1990).
4. Glutathione (GSH): GSH level was estimated by method of Beuter *et al*. (1963).

Metal analysis by atomic absorption spectrometry :

The levels of Cu, Zn, Mn, Al and Se in plasma were determined by atomic absorption spectrometry according to the method of Taylor and Bryant (1981).

Statistical analysis

The results are expressed as means ± SD. Statistical analysis was performed according to the method of Murray (1982). Data were analyzed using unpaired Student's t-test. P values of < 0.05 were considered to be statistically significant.

RESULTS

Data concerning the hemodialysis and control groups are shown in Table 1. In pre-hemodialysis patients, the levels of MDA, CAT and SOD were higher and GSH level was lowered as compared to that value of controls. While MDA, CAT and SOD levels were higher in patients after hemodialysis than that of control group. In contrast, there is no significant difference in the level of GSH between the control and post-hemodialysis groups. The level of GSH of post-hemodialysis patients was higher than its level before the dialysis session. In those groups, levels of MDA, CAT and SOD were not significantly different in post-dialysis group compared to that value before dialysis. The mean plasma levels of trace elements in all groups and standard deviations are shown in Table 2. This Table shows that there was no significant difference in (Se) level between controls and the pre-hemodialysis values. On the other hand, the levels of Cu, Zn and Mn were lowered in patient pre-dialysis than that the value of control group. The level of Al in the pre-dialysis group showed a significant elevation when compared to the controls but no significant changes occurred between groups before and after dialysis process. After hemodialysis, the levels of Se, Cu, Zn and Mn were highly significant decrease compared to that value compared to control group. No significant changes were observed in the levels of the studied trace elements in pre-hemodialysis patients compared to their levels after dialysis.

TABLE I: Mean and standard deviation of erythrocytes SOD and CAT activities as well as MDA and GSH contents in plasma of CRF patients and healthy control group.

Parameters	Control group (n=11)	Group 1 (n=12)	Group 2 (n=13)
MDA (nmole/ml)	10.13 ± 2.54	14.31 ± 0.21**	15.24 ± 0.17^{++}
SOD (U/g Hb)	1504 ±79	2089 ±78**	2117 ± 45^{++}
CAT (U/g Hb)	2.14 ± 2.34	3.71 ±1.02**	4.28 ± 0.92^{++}
GSH (µmol/ml)	18.13 ± 1.67	14.19 ± 3.34**	19.28 ± 2.14^{NS}

** P <0.001 and $^{++}$P < 0.001 vs. control group

TABLE II: Mean and standard deviation of plasma trace element in CRF patients and healthy control group

Element (µmole/l)	Control group (n=11)	Group 1 (n=12)	Group 2 (n=13)
Se	2.14 ± 0.07	1.80±0.012^{NS}	1.79 ±0.003^{NS}
Al	4.02 ± 0.05	6.07 ±0.520**	5.62 ±1.17^{NS}
Cu	25.03 ± 0.05	20.82±0.073**	21.16±0.072^{++}
Mn	32.18 ± 0.24	18.0±0.057**	17.6 ±0.054^{++}
Zn	102 ± 12.02	85.0±0.05**	84.3 ±0.153^{++}

** P <0.001 and $^{++}$P < 0.001 vs. control group

DISCUSSION

Hemodialysis patients are at risk for deficiency of essential trace elements and excess of toxic trace elements, both of which can affect heath. Though trace elements occur in very low concentrations in the body, their role in the maintenance of undisturbed biological functions is nonetheless highly important (Tonelli *et al*., 2009). During dialysis some trace elements can accumulate in the body because of dialysis fluid impurities and others may remove from blood to dialysate leading to deficiency of some trace elements in the body (Esfahani *et al*., 2007). In CRF, the concentrations of trace elements are modified as a consequence of endogenous toxicities and of impaired renal function, partly due to dietary restriction and therapeutic measures (Menevse *et al*., 2006). Our study showed that there is no significant changes of plasma MDA and erythrocyte SOD, CAT and highly significant increase in the level of plasma GSH in the post dialysis group when compared with the pre-dialysis group. These finding are in agreement with the previous studies which have reported on elevation of lipid peroxidation in CRF patients and together with dialysis was associated with an impairment of antioxidant defense and overproduction of oxidative stress markers (Chugh *et al*., 2000 , Drai *et al.,* 2001, Reed *et al.,* 2008). Whereas some researchers have concluded that zinc and selenium deficits in uremic patients were correlated with disturbance in the enzyme systems which detoxify free radicals (Richard *et al*., 1991). Oxidative damage can be caused by the imbalance between the production of free radicals and the countering

effect of various antioxidant enzymes (Massy and Nguyen-Khoa, 2002). In this respect concerning selenium levels in control and post-hemodiaysis patients are consistent with the results presented by (Bogye *et al*., 2000 , Zaxhara *et al.,* 2006) who found there was no significant difference between serum selenium concentration before and after dialysis session. Neiva *et al.* (2002) stated that decreased element concentrations are related mainly to nutritional intake, intestinal uptake and altered distribution and in addition that protein bound trace elements may be lost more in the presence of proteinuria. Furthermore, uremic compounds may be related to accumulation of aluminium. According to the present data the levels of aluminium are higher in hemodialysis patients than in control group which are consistent with those studies (Vanholder *et al*., 2002). Moreover, this study showed that there is significant decrease in the concentration of Zn, Cu, Mn in pre-dialysis group compared to the control which are in agreement to finding of Pietrzak *et al*. (2002). Zinc deficiency leads to immune deficiency and susceptibility to infections (Fischer and Black, 2004, Batista *et al.,* 2006). Moreover, the deficiency of manganese may cause impaired growth, abnormal metabolism of glucose and lipids (Zima *et al.,* 1999). In conclusion, our finding suggests that in CRF patients undergoing hemodialysis, oxidant and antioxidants play a vital role in the pathogenesis of disease. Thus, the increased reactive oxygen species (ROS) accompanied by decreased antioxidant defense in CRF patients on hemodialysis and increased lipid peroxidation in erythrocyte membranes. Moreover, according to the current results of aluminium are statistically different between groups which point out that it may be important to analyze this element. The abnormal metabolism of aluminum may contribute to a part of hemodialysis.

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