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Effect of Haemodialysis on Plasma Lipid Peroxidation and Endogenous Non-enzymic Antioxidants in Gorgan (South East of Caspian Sea)

Abdoljalal Marjani

The aim of this study was to evaluate the effect of haemodialysis on lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde) and endogenous non-enzymic antioxidants before and after the dialysis and compared with control group. The sampling procedure was a purposive sampling. Twenty two patients with Chronic Renal Failure (CRF) disease who were haemodialysed at 5th Azar hospital of Gorgan Dialysis Center (2005). Twenty two age and sex matched healthy control were recruited for this study. Plasma level of malondialdehyde and uric acid were significantly increased and reduced in the postdialysis group when compared with predialysis and control group, respectively ($p < 0.001$). The plasma level of albumin and total bilirubin were significantly increased in postdialysis group when compared with predialysis group ($p < 0.001$). The increasing level of plasma lipid peroxidation and the significant difference of non-enzymic antioxidants in the haemodialyzed patients after the process of dialysis, maybe related with the patient uremia, the quality of consumed water in the dialysis procedure, dialysis membrane and the loss of these antioxidants through membranes and hemoconcentration of them during the dialysis process and the dialysis process (may increase lipid peroxidation during the dialysis process). These states of affairs may play an important role in progress of cardiovascular abnormality in haemodialyzed patients. Due to this conditions a review of haemodialysis membrane, the techniques used in the dialysis, the consumption of various oral antioxidant, the elimination of active oxygens from the dialysis surrounding are among the measures which can prevent sudden cardiovascular abnormality in the haemodialysis patients and ultimately these important factors up- grade the patients quality of life and prevent sudden silent myocardial infarction.

Key words: Haemodialysis, lipid peroxidation, endogenous nonenzymic antioxidants

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INTRODUCTION

Free radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism and in the course of free radical mediated diseases such as cancer, diabetes mellitus, cardiovascular and renal diseases (Kohen *et al.*, 1996). Patients with chronic renal failure, including those receiving regular long-term haemodialysis have a high incidence of premature cardiovascular disease (Loughrey *et al.*, 1994). Free radicals may cause lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde) and damage macromolecules and cellular structure of the organism, endothelium and erythrocytes. Plasma malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to definite oxidation of polyunsaturated fatty acids such as linoleic and linolenic acid and thus serves as a reliable marker of lipid peroxidation (Boaz *et al.*, 1999; Fiorillo *et al.*, 1998). Plasma MDA is a predictor of cardiovascular disease in patients on haemodialysis, which may underscore the role of oxidative stress as a cardiac risk factor in these patients (Boaz *et al.*, 1999b). Some studies have shown that haemodialysis is connected with increased free radical production (Bast *et al.*, 1991). Cardiovascular disease is one of the leading cause of death in chronic renal failure patients on dialysis, as well as leading cause morbidity (Raine *et al.*, 1992). Clinical and subclinical myocardial ischaemia are common among chronic renal failure patients, both before and during dialysis (Folay *et al.*, 1995; Singh *et al.*, 1994). The prevalence of ischaemic heart disease in haemodialysis patients in 10-20 times higher than that in the general population with 50% mortality due to Cardiovascular disease. According to the US Renal Data System 42% of patients undergoing haemodialysis have had a myocardial infarction or Coronary revascularization. In addition, the rate of survival after myocardial infarction is much lower for haemodialysis patients than for the general population (Heeschen *et al.*, 2000). Free radicals are eliminated from the body by their interaction with non-enzymic and enzymic antioxidants such as uric acid, albumin, bilirubin, vitamins E, C, A, glutathione, glutathione peroxidase, superoxide dismutase and catalase. There are a few reports describing differences in plasma lipid peroxidation and non-enzymic antioxidants between haemodialysis patients and controls. Some studies show increasing, some others show decreasing and no any changes of plasma lipid peroxidation and non-enzymic antioxidants. Non-enzymic antioxidants such as uric acid, albumin and bilirubin are low molecular weight antioxidants which is important when enzymic antioxidants are in low levels. Because of the contradictory results in this field of study, the present study was designed to determine the effect of haemodialysis on plasma lipid peroxidation (by measuring

the level of MDA) and endogenous non-enzymic antioxidants (uric acid, albumin and total bilirubin) before and after the dialysis process at a Haemodialysis Center in Gogan City in comparison to controls.

MATERIALS AND METHODS

The samples were obtained in a purposive fashion, from 22 haemodialysis patients (mean age 43.54 ± 9.21 years of old) and control subjects (mean age 43.77 ± 9.33 years of old). The mean length of dialysis for each patients was 3.95 ± 0.14 h with average 2.27 ± 0.45 times a week. The patients studied had no evidence of vascular complications, including hypertension, coronary artery disease. Controls were defined as not having a major medical illness, no hospital admissions, no current medication and a subjective perception of good health as determined by health questionnaire. None of the study subjects received any medical (vitamine E,C) supplement and non-medical antioxidants (tomato, orange, etc.). Patients were chosen (14 male, 8 female) from the patients referred to the Department of Haemodialysis Center at the 5th azar hospital in Gorgan City. This study was carried out during 2005. Blood samples were obtained after an overnight fast in heparinized tubes just before and after the process of dialysis and control group. Plasma was separated soon after blood was taken. The plasma urea, creatinine, malondialdehyde (the level of lipid peroxidation expressed as malondialdehyde [MDA]), uric acid, albumin and total bilirubin were determined using laboratory kits and spectrophotometry techniques (model JENWAY 6105 UV/VIS) in the laboratory of biochemistry (Faculty of Medicine). Plasma malondialdehyde was determined with Kei Satoh method (Satoh, 1978). Plasma urea, creatinine, uric acid, albumin and total bilirubin were determined using previously described methods (Fabiny and Erthinghausen, 1971; Henry *et al.*, 1957; Doumas and Watson, 1971; Kaplan *et al.*, 1998). Data was analyzed by Student's t-test, using SPSS-10 software. $p < 0.05$ was considered significant.

Malondialdehyde measurement : To 0.5 mL plasma, 2.5 mL of trichloroacetic acid was added and the tube was left to stand for 10 min at room temperature. After centrifugation at 3500 RPM for 10 min, the supernatant was decanted and the precipitate was washed once with sulfuric acid. The after, 2.5 mL sulfuric acid and 3 mL thiobarbituric acid (TBA) in sodium sulfate were added to the precipitate and the coupling of lipid peroxide with TBA was carried out in a boiling water bath for 30 min. After cooling in cold water, the resulting chromogen was extracted with 4 mL of n-butyl alcohol by vigorous shaking. Separation of the organic phase was facilitated by centrifugation at 3000 RPM for 10 min and its absorbance was determined at the wavelength of 530 nm.

Table 1: The mean and standard deviation of plasma urea, creatinine, uric acid, albumin, total bilirubin and malondialdehyde

Test	Predialysis	Postdialysis	Control	p-value
Urea (mg dL ⁻¹)	123.54±8.51	55.68±7.96	26.37±4.83	<0.001
Creatinine (mg dL ⁻¹)	15.88±3.07	1.96±0.45	1.08±0.29	<0.001
Uric acid (mg dL ⁻¹)	6.1±1.06	2.89±0.93	4.25±0.64	<0.001
Albumin (g dL ⁻¹)	3.42±0.63	3.78±0.51	3.83±0.36	<0.001*
Total bilirubin (mg dL ⁻¹)	0.65±0.21	0.75±0.11	0.77±0.35	<0.001**
Malondialdehyde (nmol mL ⁻¹)	1.27±0.23	2.32±0.38	0.98±0.17	<0.001

There was no significant differences between the postdialysis and control group*, There was no significant differences between the dialysis and control group**

RESULTS

As shown in Table 1 plasma level of malondialdehyde showed significant difference between predialysis and control group (p<0.001). It was significantly increased in the postdialysis group when compared with predialysis and control group (p<0.001, p<0.001). The plasma level of uric acid was significantly reduced in the postdialysis group when compared with predialysis and control group (p<0.001, p<0.001). There was also significant difference between control and predialysis group (p<0.001). The plasma level of albumin was significantly increased in postdialysis group when compared with predialysis group (p<0.001). It was also significant difference between predialysis and control group (p<0.001). There was no significant difference between the postdialysis and control group (p>0.05). The plasma level of total bilirubin was significantly increased in the postdialysis group when compared with the predialysis group (p<0.001). There was no significant differences between the (pre-, post-) dialysis and control group (p>0.05).

DISCUSSION

The aim of the present study was to determine the plasma level of malondialdehyde and endogenous non-enzymic antioxidants in predicting the outcome of haemodialysis patients on regular dialysis. There are a few reports describing difference in plasma lipid peroxidation and endogenous non-enzymic antioxidants in haemodialyzed patients. Some of the studies showed an increase while some other showed a decrease or no significant differences. Increasing amount of free radicals probably could lead to the reduction of number of nephrons, glomerol filtration rate and also paranchymal lesions. The free radical also can cause the membrane lipid peroxidation, glomerul and renal tubules damage (Trachman *et al.*, 1992). The results of this study show that the plasma level of malondialdehyde was significantly increased in postdialysis group when compared with predialysis and control groups.

Canestrari *et al.* (1995) reported that the level of plasma malondialdehyde in haemodialyzed patients was higher than healthy controls.

Study of Samouilidou and Grapsa (2003) on 31 haemodialysis patients and 17 control group showed that plasma malondialdehyde of haemodialysis patients increased in the predialysis group when compared with postdialysis group. But the level of plasma malodialdehyde was higher in control groups when compared with postdialysis group.

Some researchers (Ozden *et al.*, 2002; Taylor *et al.*, 1992; Toborek *et al.*, 1992; Balashova *et al.*, 1992) reported that the level of plasma malondialdehyde in hsaemodialysis patients increased when compared with control groups.

In this study we determined the level of plasma malodialdehyde of haemodialysis pateints before and after the dialysis process. Present results show a significant increase of plasma malondialdehyde in the postdialysis group when compared with the predialysis group. There was significant difference between the predialysis and postdialysis group. Present results are in agreement with the groups mentioned in that the plasma level of malondialdehyde of haemodialysis patients is significantly increased from that of controls (Loughery *et al.*, 1994; Ozdne *et al.*, 2002; Taylor *et al.*, 1992; Toborek *et al.*, 1992; Balashova *et al.*, 1992). But the results of this study are not in agreement with the results of Samouilidou and Grapsa (2003) showing plasma of malondialdehyde of haemodialysis patients were significantly decreased after the dialysis process. This situation probably in due to direct relation between the blood of haemodialysis patients with dialysis instrument, which is an conductive factor in oxidative stress and subsequent increased production of free radicals in haemodialysis patient. The probable Oxidative destruction can be due to increasing production of free radicals (Hussain *et al.*, 1995; Dasgupta *et al.*, 1992). This study provides evidence that increased lipid peroxidation in haemodialysed patients is related to the dialysis process treatment rather than the disease itself. This situation may play a role in the development of atherosclerosis in haemodialysed groups. There are a few studies on changes in plasma endogenous non-enzymic antioxidants. Yilmaz *et al.* (2003) and Wratein *et al.* (2000) reported that plasma uric acid of haemodialysis patients decreased in the postdialysis group when compared with predialysis group. But palsa uric acid was higher in the predialysis group when compared with control group. Yilmaz *et al.* (2003) reported that plasma total bilirubin of haemodialysis patients did not show any significant differences in the pre- and postdialysis groups. But there was a meaningful increasing of plasma total bilirubin in the postdialysis group when compared with the predialysis group.

Plasma albumin of haemodialysis patients did not show any significant differences in the postdialysis group when compared with control group. But plasma albumin

showed a meaningful increasing in the postdialysis and control groups when compared with the predialysis group (Yilmaz *et al.*, 2003).

Study of Malliaraki *et al.* (2003) have shown that uric acid and albumin concentrations decrease and gradual increase during the dialysis process, respectively. In contrast, minor changes of total bilirubin were found during the dialysis process. Study of Gonenc *et al.* (2002) have shown that uric acid and albumin levels increase and decrease during the dialysis process respectively. In contrast, there was not any changes of total bilirubin during dialysis process. The results of our study showed a meaningful decreasing of plasma uric acid between pre- and postdialysis group is thought to be related with the loss of antioxidant through the membranes and the decreased plasma uric acid maybe related to increasing of lipid peroxidation in haemodialyzed patients. Plasma albumin and total bilirubin were increased in the postdialysis group when compared with predialysis group. There were no significant differences between the dialysis and control group. Plasma albumin and total bilirubin were increased in the dialysis group probably due to the hemoconcentration during the dialysis (Malliaraki *et al.*, 2003). Albumin can react with most oxygenated species, which could lead to some oxidation-induced changes. A study showed that the antioxidant effect of albumin could result from its high content in cysteine residues included in thiolate clusters and various amino acid residues (Tyr, Trp, Met, Lys and Arg (Meucci *et al.*, 1998). The thiolate groups of the protein contribute physiologically to the redox balance and modulate oxidative stress (Hultqvist *et al.*, 1997). In this study our results are in agreement (Yilmaz *et al.*, 2003; Wrattein *et al.*, 2000; Malliaraki *et al.*, 2003) with the groups mentioned in that the plasma level of malondialdehyde (increasing level of MDA) and endogenous non-enzymic antioxidants (decreasing level of uric acid and increasing level of albumin and total bilirubin in dialysis group but not a meaningful increase when compared with control groups) of haemodialysis patients is significantly different from that of control. But our results are not in agreement with the other studies (Malliaraki *et al.*, 2003; Gonenc *et al.*, 2002). Different explanations for these results could be suggested:

- Water elimination during dialysis causes increased concentration of endogenous antioxidant substances (Meucci *et al.*, 1998).
- Elimination of uric acid modifies the equilibrium between oxidized and reduced status of endogenous and exogenous antioxidants (Canestrari *et al.*, 1995; Hultqvist *et al.*, 1997).
- The elimination of water-soluble metabolic antioxidants (uric acid) modifies the equilibrium of lipid and water-soluble antioxidants (Barbaste *et al.*, 2002).

The observation of meaningful increasing level of plasma lipid peroxidation and the significant difference of non-enzymic antioxidants between pre- and postdialysis group in the haemodialyzed patients after the process of dialysis, maybe related with the patient uremia, the quality of consumed water in the dialysis procedure, dialysis membrane and the loss of these antioxidants through membranes and hemoconcentration of them during the dialysis process and the dialysis process self (may increase lipid peroxidation during the dialysis process). These states of affairs may play an important role in progress of cardiovascular abnormality in haemodialyzed patients. Due to this conditions a review of haemodialysis membrane, the techniques used in the dialysis, the consumption of various oral antioxidant, the elimination of active oxygens from the dialysis surrounding are among the measures which can prevent sudden cardiovascular abnormality in the haemodialysis patients and ultimately these important factors up- grade the patients quality of life and prevent sudden silent myocardial infarction.

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