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Clinical Effect of Haemodialysis on Plasma Lipid Peroxidation and Cardiac Troponin I in Gorgan (South East of Caspian Sea)

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Abstract: The aim of this study with the discriminative information was to evaluate the effect of haemodialysis on plasma lipid peroxidation and Cardiac Troponin I before and after the dialysis process. Twenty two patients with Chronic Renal Failure (CRF) disease who were haemodialyzed at 5th Azar hospital of Gorgan Dialysis Center were recruited for this study (2005). Plasma malondialdehyde was increased significantly in the postdialysis group when compared with predialysis. Plasma levels of Cardiac Troponin I in 12 haemodialyzed patients were significantly increased in the postdialysis group when compared with predialysis, whereas plasma level of Cardiac troponin I in 10 haemodialyzed patients showed less than $0.1 \mu\text{g L}^{-1}$ which is in normal range. The observation of meaningful increasing level of plasma lipid peroxidation and Cardiac Troponin I in the haemodialyzed patients after the process of dialysis, maybe related with the patient uremia, dialysis membrane 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, Cardiac Troponin I

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.*, 1999a, b; 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.*, 1999a, b). Some studies have shown that haemodialysis is connected with

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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 (Foley *et al.*, 1995; Singh *et al.*, 1994). Earlier detection of cardiovascular abnormality in these patients might allow earlier interventions to reduce morbidity and mortality. The prevalence of ischaemic heart disease in haemodialysis patients is 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). Cardiac Troponin I is specific marker of myocardial damage (Bodor *et al.*, 1992; Coudry, 1998). Patients with chronic renal failure undergoing haemodialysis have a high incidence of Cardiac events (Foley *et al.*, 1995) and of false-positive increases in myoglobin as well as creatine kinase and its MB isoenzymes (Jaffe *et al.*, 1984; Pierce and Jaffe, 1986). Cardiac Troponin I may be increased in these patients without evidence of ischaemic myocardial damage (McLaurin *et al.*, 1997). In haemodialysis patients, the clinical symptoms of Cardiac damage are difficult to diagnosis and may be deceptive. In addition, high serum concentrations of myoglobin and creatine kinase-MB lack specificity. In recent years, Cardiac Troponin I has been increasingly used in the diagnosis of acute coronary syndromes as studies have shown their greater clinical sensitivity over creatine kinase-MB (D'Costa *et al.*, 1997).

The aim of this study with the discriminative information was to evaluate the effect of haemodialysis on plasma lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde) and Cardiac Troponin I before and after the dialysis process, to find out the effect of haemodialysis on the plasma levels of lipid peroxidation and Cardiac Troponin I.

Materials and Methods

The sampling procedure was purposive sampling which carried out on 22 haemodialysis patients (without symptoms of myocardial ischaemia) with average age 43.54±9.21 years of old (range 21-55). The mean length of dialysis for each patients was 3.95±0.14 h with average 2.27±0.45 times a week. Neither of them received antioxidant medicines and foods. Patients were chosen (14 male, 8 female) from the patients referred to the Department of Haemodialysis Center at 5th Azar hospital in Gorgan University of Medical Sciences (2005). The patients studied had no evidence of vascular complications, including hypertension, coronary artery disease.

Blood samples were obtained from the patients just before and after the dialysis process of dialysis in a heparinized tubes. Plasma is separated as soon as the blood taken. The plasma urea, creatinine, lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde (MDA)) were determined for haemodialyzed patients before and after the dialysis process, using laboratory kit spectrophotometry technique (model JENWAY 6105 UV/VIS) in the laboratory of Biochemistry (faculty of medicine). Plasma malondialdehyde was determined with Kei Satoh method (Satoh, 1978). Cardiac Troponin I was determined on the VIDAS instrument (made in FRANCE) using the ELFA (Enzyme-Linked Fluorescent Assay) technique (Adams *et al.*, 1993).

Data was analyzed by student's t-test using spss-11.5 software. p-value less than 0.05 was considered significant.

Malondialdehyde Measurement

To 0.5 mL plasma, 2.5 mL of trichloroacetic acid is added and the tube is left to stand for 10 min at room temperature. After centrifugation at 3500 rev min⁻¹ for 10 min, the supernatant is decanted and the precipitate is washed once with sulfuric acid. Then 2.5 mL sulfuric acid and 3 mL thiobarbituric

acid (TBA) in sodium sulfate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling water bath for 30 min. After cooling in a cold water, the resulting chromogen is extracted with 4 mL of n-butyl alcohol by vigorous shaking. Separation of the organic phase is facilitated by centrifugation at 3000 rev min⁻¹ for 10 min and its absorbance is determined at the wavelength of 530 nm.

Results

In present study we determined the plasma levels of malondialdehyde and Cardiac Troponin I in 22 patients with Chronic Renal Failure. As shown in Table 1 plasma level of malondialdehyde and Cardiac Troponin I (in 12 haemodialyzed patients) were significantly increased after the dialysis process when compared with the predialysis (p<0.001). The plasma levels of Cardiac Troponin I in 12 haemodialyzed patients were significantly increased before the dialysis process when compared with normal range (p<0.001). The plasma levels of Cardiac Troponin I in 10 haemodialyzed patients were in normal range (less than 0.1 µg L⁻¹). The ranges of >0.1-<0.8 µg L⁻¹ show possible myocardial damage. The ranges of >0.8 µg L⁻¹ show acute myocardial Infarction Cut-off.

Table 1: The mean and standard deviation of plasma urea, creatinine, malondialdehyde and Cardiac Troponin I

| Test | Predialysis | Postdialysis | p-value |
|---|-------------|--------------|---------|
| Urea (mg dL ⁻¹) | 123.54±8.51 | 55.68±7.96 | <0.001 |
| Creatinine (mg dL ⁻¹) | 15.88±3.07 | 1.96±0.45 | <0.001 |
| Malondialdehyde (nmol mL ⁻¹) | 1.30±0.22 | 2.35±0.38 | <0.001* |
| Cardiac troponin I (n = 12) (µg L ⁻¹) | 0.21±0.040 | 0.57± 0.09 | <0.001* |
| Cardiac troponin I (n = 10) (µg L ⁻¹) | <0.1 | <0.1 | >0.001 |

*p-value was significant

Discussion

The aim of the present study was to determine the plasma level of malondialdehyde and Cardiac Troponin I in predicting the outcome of haemodialysis patients on regular dialysis. There are a few reports describing difference in plasma lipid peroxidation and Cardiac Troponin I 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, glomerular filtration rate and also parenchymal lesions. The free radical also can cause the membrane lipid peroxidation, glomerular and renal tubules damage (Trachman *et al.*, 1992). The results of this study show that the plasma level of malondialdehyde and Cardiac Troponin I were significantly increased in postdialysis group when compared with predialysis group.

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 (Loughrey *et al.*, 1994; Ozden *et al.*, 2002; Taylor *et al.*, 1992; Toborek *et al.*, 1992; Balashova *et al.*, 1992) reported that the level of plasma malondialdehyde in haemodialysis patients increased when compared with control groups.

In our study we determined the level of plasma malodialdehyde of haemodialysis patients before and after the dialysis process. Our 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 (Loughrey *et al.*, 1994; Ozden *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; Sanaka *et al.*, 1995).

There are a few studies describing difference in plasma lipid peroxidation and Cardiac Troponin I in haemodialyzed patients without myocardial ischaemia symptoms. The diagnosis of myocardial ischaemia is difficult in haemodialysis patients since they are not able to perform adequate exercise tests due to limited exercise tolerance. Some studies showed that the level of Cardiac Troponin I increased in haemodialyzed patients without myocardial ischaemia symptoms (Adams *et al.*, 1993; Khan *et al.*, 2001; Beciani *et al.*, 2003). Some other studies reported that the level of Cardiac Troponin I show no significant differences before and after the dialysis process in haemodialyzed patients (Tun *et al.*, 1998; Donnino *et al.*, 2004). Our results are in agreement with the results of studies showing that the the plasma level of Cardiac Troponin I increased in haemodialyzed patients (12 haemodialyzed patients) after the dialysis process (Adams *et al.*, 1993; Khan *et al.*, 2001; Beciani *et al.*, 2003). But the results of this study are not in agreement showing that the plasma level of Cardiac Troponin I in haemodialyzed patients showed no significant differences (Donnino *et al.*, 2004) after the dialysis process.

The observation of meaningful increasing level of plasma lipid peroxidation and Cardiac Troponin I in the haemodialyzed patients after the process of dialysis, maybe related with the patient uremia, dialysis membrane 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.

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