



Asian Journal of
Cell Biology

ISSN 1814-0068



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Effect of Dialysis on Plasma Lipid Peroxidation and Lipid Profile in Haemodialysis Patients

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Abstract: This research has been set up to study the possibility of lipid peroxidation and lipid profile variation in haemodialysis patients. 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). The patients don't have coronary heart disease. 22 age and sex matched healthy control were recruited for this study. Plasma levels of lipid peroxidation and lipid profile were determined in haemodialysis patients and compare with control groups. The results from this research projects indicate that Plasma levels of lipid peroxidation (1.27 ± 0.23 nmol mL⁻¹) and triglyceride (195.22 ± 20.28 mg dL⁻¹) in haemodialysis patients were significantly increased when compared with control subjects (0.98 ± 0.17 nmol mL⁻¹, 151.09 ± 5.74 mg dL⁻¹, respectively), whereas plasma level of total cholesterol (171.68 ± 16.30 mg dL⁻¹), High Density Lipoprotein cholesterol (HDL-C, 44.13 ± 5.38 mg dL⁻¹) in haemodialyzed patients showed no significant difference when compared with control subjects (170.77 ± 14.73 and 44.77 ± 5.29 mg dL⁻¹, respectively). The changes in level of plasma lipid peroxidation and triglyceride in haemodialyzed patients, maybe related with the patient uremia, dialysis membrane and the dialysis process. The metabolic alterations accompanying renal failure may be responsible for dyslipidemia independent of the fat distribution in haemodialysis patients. 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 oxygen from the dialysis surrounding are among the measures which can prevent sudden cardiovascular abnormality in the haemodialysis patients.

Key words: Haemodialysis, lipid peroxidation, lipid profile

Introduction

Heart disease is a major cause of morbidity and mortality among patients with renal failure. Mortality in dialysis patients is dramatically higher than in the general population and cardiovascular disease is the leading cause of mortality among these patients (Foley *et al.*, 1998). Chronic renal failure is often associated with dyslipidemia. Lipid profile abnormalities has been identified as an independent risk factor for atherosclerosis (Green *et al.*, 1983; Jeppesen *et al.*, 1998; Assman and Schulte, 1992; Hokanson and Austin, 1996). Some of them persisting and becoming worse during dialysis treatment.

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; 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). 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). 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).

Dyslipidemia and oxidative stress may be the main atherogenic risk factors in chronic renal failure (Chan *et al.*, 1981).

For this reason 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 lipid profile (total cholesterol, High Density Lipoprotein cholesterol (HDL-C) and triglyceride in haemodialysis patients and compare with control groups.

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 during fasting in a heparinized tubes. Plasma is separated as soon as the blood taken. The plasma total cholesterol, High Density Lipoprotein cholesterol (HDL-C) and triglyceride (TG) were determined for haemodialyzed patients, using routine laboratory kit specterophotometry technique (model JENWAY 6105 UV/VIS) in the Laboratory of Biochemistry (Faculty of Medicine). The plasma lipid peroxidation (the level of lipid peroxidation expressed as malondialdehyde (MDA) was determined with Kei Satoh (1978) method. 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 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 for 10 min and its absorbance is determined at the wavelength of 530 nm.

Results and Discussion

Plasma levels of malondialdehyde and lipid profile of 22 haemodialyzed patients and control subjects are reported in Table 1. There are a tendency to higher triglyceride and malondialdehyde levels in haemodialyzed patients when compared to control subjects. The difference is statistically significant. It was no significant difference of total cholesterol and HDL cholesterol between haemodialyzed patients and control subjects.

Several treatment approaches are currently proposed or are under investigation in order to decrease the burden of cardiovascular disease in the haemodialysis patients. Cardiac mortality is the greatest contributor to overall mortality in this susceptible population, vigorous efforts are required to identify and correct potential factors that may exacerbate this problem. The aim of the present study was to determine and compare the plasma levels of malondialdehyde and lipid profile (total cholesterol, High Density Lipoprotein Cholesterol (HDL-C) and triglyceride (TG) in predicting the outcome of haemodialysis patients on regular dialysis.

There are a few reports describing difference in plasma lipid peroxidation and lipid profile in haemodialyzed patients. Some of the studies showed an increase while some other showed a decrease or no significant differences. This study has demonstrated that plasma levels of malondialdehyde and triglyceride were significantly higher in the haemodialysis patients than in the control subjects, while the plasma levels of total cholesterol and High Density Lipoprotein Cholesterol (HDL-C) showed no significant differences in both groups. Canestrari *et al.* (1995) reported that the level of plasma malondialdehyde in haemodialyzed patients was higher than healthy controls. Study of Samouilidou *et al.* (2003) on 31 haemodialysis patients and 17 control group showed that plasma malondialdehyde of haemodialysis patients decreased when compared with control groups. Some researchers (Loughrey *et al.*, 1994; Canestrari *et al.*, 1995; 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. The present show a significant increase of plasma malondialdehyde in haemodialysis patients when compared with control groups. 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; Canestrari *et al.*, 1995; Ozden *et al.*, 2002, 1992; Toborek *et al.*, 1992; Balashova *et al.*, 1992). But the results of this study are not in agreement with the results of Samouilidou *et al.* (2003) showing plasma of malondialdehyde of haemodialysis patients were significantly decreased. 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 patients. 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). An oxidative stress due to overproduction of reactive oxygen species by activated monocytes and impairment in antioxidant defence mechanisms (Loughrey *et al.*, 1994; Paul *et al.*, 1993; Saint-Georges *et al.*, 1989) may be take place in haemodialysis patients (Cristol *et al.*, 1994; Himmelfarl *et al.*, 1991) and may contribute to the accelerated haemodialysis-induced atherogenesis by oxidatively modified proteins and lipids. There

Table 1: The plasma levels of total cholesterol, Triglyceride, HDL cholesterol and Malondialdehyde in haemodialysis patients and control groups

| Test | Haemodialysis patients | Control | p-value |
|------------------------------------------|------------------------|--------------|---------|
| Total cholesterol (mg dL ⁻¹) | 171.68±16.30 | 170.77±14.73 | *NS |
| Triglyceride (mg dL ⁻¹) | 195.22±20.28 | 151.09±5.74 | *<0.001 |
| HDL cholesterol (mg dL ⁻¹) | 44.13±5.38 | 44.77±5.29 | *NS |
| Malondialdehyde (nmol mL ⁻¹) | 1.27±0.23 | 0.98±0.17 | *<0.001 |

NS = Not significant
*p = significant

are a few studies describing difference in plasma lipid peroxidation and lipid profile 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.

Lee *et al.* (1997) suggested that the uraemic condition affected lipid abnormality. Odamaki *et al.* (1999) showed that the presence of renal failure would modify lipid metabolism by altering the fat distribution. Prichard *et al.* (2003) showed that plasma levels of triglyceride and malondialdehyde in patients who undergo haemodialysis are increased while HDL-cholesterol are decreased.

Sharma *et al.* (1980) reported that hypertriglyceridemia was observed in only 2 out of 19 cases on dialysis. Ibels *et al.* (1976) also reported decrease in hypertriglyceridemia after initiation of dialysis. This may be due to improved triglyceride removal by increased post heparin lipolytic activity and decreased peripheral resistance to insulin after initiation of dialysis. Moberly *et al.* (2002) showed that plasma level of total cholesterol and triglyceride increased in haemodialysis patients when compared with control groups, while HDL-cholesterol decreased in these patients. Present results are in agreement with the groups mentioned in that the plasma level of triglyceride of haemodialysis patients is significantly increased from that of controls (Prichard, 2003; Moberly *et al.*, 2002). But the results of this study are not in agreement with the results of some researchers showing plasma of triglyceride of haemodialysis patients were significantly decreased (Ibels *et al.*, 1976) or not changed (Sharma *et al.*, 1980). The results of this study also are not in agreement with the groups showing plasma of HDL-cholesterol of haemodialysis patients were significantly increased (Prichard, 2003) and decreased (Moberly *et al.*, 2002).

The observation of meaningful increasing level of plasma lipid peroxidation and triglyceride in the haemodialyzed patients, maybe related with the patient uraemia, dialysis membrane and the dialysis process (may increase lipid peroxidation during the dialysis process). The metabolic alterations accompanying renal failure may be responsible for dyslipidemia independent of the fat distribution in haemodialysis patients. Haemodialysis patients have been reported to have a decreased concentration of hepatic lipase (Arnadottir *et al.*, 1995) and an increased level of the lipoprotein lipase inhibitor (Moberly *et al.*, 1999). 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.

Acknowledgments

This research was supported by research deputy of Golestan University of Medical Sciences. I acknowledge with thanks the help of Research and Biochemistry Departments.

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