Evaluation of Oxidative Stress in Blood During Hemodialysis

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ABSTRACT

The number of patients with end-stage renal disease is growing rapidly in Asia. Patients with end stage renal disease failure (ESRD) undergoing long term hemodialysis (HD) are at risk for high oxidative stress. There is paucity of information with regard to oxidative stress during HD. Therefore, the aim of the present study was to evaluate catalase enzyme activity in erythrocytes and the total antioxidant capacity (TAC) in plasma before, during and after dialysis in hemodialysis patients. The cross-sectional study carried out in thirty four patients (16 males and 18 females) having mean age of 55 ± 16 years undergoing HD three times per week were included. The enzyme activity of catalase was measured by UV Spectrophotometric method and total antioxidant capacity in plasma were observed by ferric reducing ability of plasma (FRAP) assay method. All tests were performed before, during and after the process of dialysis. The prevalence of erythrocyte catalase enzyme activity was observed to be significantly decreased ($P < 0.001$) during (107.01 ± 36.2) and after (105.30 ± 36.75) dialysis as compared to its activity before (159.75 ± 30.98) dialysis. On the other hand, a total antioxidant capacity in plasma was noticed to be significantly elevated ($P < 0.001$) during (648.52 ± 14.66) and after (573.08 ± 15.16) dialysis as compared to before (486.61 ± 14.86) dialysis. The study concluded that dialysis process affects erythrocyte catalase enzyme activity as well as total antioxidant capacity in plasma of patients. HD exacerbates oxidative stress and disturbances in antioxidant enzymes in patients.

Keywords: Catalase, Total antioxidant, Hemodialysis, Oxidative stress

INTRODUCTION

The number of patients with end-stage renal disease is growing rapidly in Asia. Data suggest that at least 2.9 million people need dialysis in Asia [1]. End stage renal failure (ESRD) occurs when the kidneys can no longer function adequately and survival depends on either dialysis or transplantation [3]. ESRD patients have a high prevalence of associated disease and low survival rates [3, 4]. Increased oxidative stress may play a role in morbidity and mortality of patients with renal failure. Oxidative stress is defined as a disturbance in the pro-and antioxidant balance in an organism [5,6]. Oxygen-free radicals and reactive oxygen species are involved in the pathogenesis of many clinical disorders by causing direct damage to lipids, proteins, and DNA or by affecting cellular signal transduction pathways [7,8]. The uraemic state and the bio-

incompatibility of HD are associated with an increased oxidative stress in HD patients [9], presumably caused by both an increased generation of oxygen-free radicals/ reactive-oxygen species and by decline in levels of different antioxidant enzymes [10]. There is increasing evidence about the presence of oxidative stress in chronic renal failure patients, and particularly in those who are subjected to hemodialysis therapy [11]. The major factors responsible includes an increase in the production of agents from oxidative metabolism (oxygen-derived substances generated by activated leukocytes, transition metal compounds, and other toxins of different molecular weight), and a decrease in anti-oxidant defence enzymes [12,13]. The oxidative stress in patients with HD is traditionally attributed to the recurrent activation of polymorph nuclear neutrophils and monocytes [14]. Hemodialysis has also been shown to increase the oxidative stress, with reactive oxygen species being generated on the surface of the dialyzer membrane like polysulfone by the activation of polymorphonuclear

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leukocytes [15]. These are activated by the concurrent effect of blood passing through the dialysis circuits, the components being activated following contact with membranes that are of poor compatibility, as well as the possible passage of dialysate endotoxins, consequent to back-filtration [16]. However, three major causes of oxidative stress in HD have been suggested Uraemic toxins, dialyser interactions and dialysate contaminants [17]. Exceeding generation of ROS resulting from activation of peripheral blood cells interacting with the dialyser membranes is proposed to be an important contributor. The dialysis membranes used in HD seem to play a central role in the increased production of oxygen free radicals in ESRD patients [18]. Even the use of low biocompatible membranes and purity of dialysis water were unable to moderate oxidative stress. The study presented here aims to measurement of activity catalase enzyme erythrocytes and level of plasma total antioxidant in HD patients with the use of polysulfone membrane [19].

PATIENTS AND METHODS

Assessment of Clinical Variables

The cross-sectional study was carried out in thirty four patients from medical and research center were included in the final analysis in 2015. Every patient with end stage renal disease on maintenance hemodialysis for 13 months or more were included after informed consent. Of these, 16 were males and 18 were females with a mean age of 55.4 ± 16.8 years having mean 16 ± 29 months of treatment undergone hemodialysis for at Department of Nephrology and Dialysis, Emam Khomeini Hospitals, in sari, Iran. The investigation conforms to the principles outlined in the Declaration of Helsinki. The study was approved by the local ethics committee and all participants gave written informed consent. Every patient had been on standard bicarbonate hemodialysis and was dialyzed three times per week for 3 h with a low-flux Polysulfone membrane with Fresenius 4008B dialysis machine (Fresenius Medical Care AG & Co. KGaA) Bad Homburg Germany [20].

Preparation of Hemolysates

2.5 ml of venous blood samples were drawn before, after and during HD from arteriovenous fistulas from patients. A total of 102 blood samples were obtained from HD patients. After an overnight fasting, in two separate tubes with EDTA. One of tubes was immediately centrifuged at 3,000 rpm, for 15 min at 4 °C, for separating erythrocytes and plasma. Erythrocytes were washed three times with saline 9% and after centrifugation at 3000 rpm, pallet diluted with cold distilled water (1:4). The lysate of erythrocyte, plasma and whole blood samples were stored at -80 °C, until analysis.

Biochemical Assay

Plasma samples were used for the measurement of Iron, Blood sugar, urea, Albumin, Triglyceride, creatinine and uric acid by Pars Azmoon laboratory kits (Pars Azmoon Co., Karaj, Iran), using an auto-analyzer (Prestige 24i, Tokyo Boeki Ltd., Tokyo, Japan).

Catalase (CAT) Activity Measurement

In the erythrocyte hemolysate catalase activity was estimated by the method of Aebi [21]. Catalase can degrade hydrogen peroxide which can be measured directly by the decrease in the absorbance at 240 nm. The hydrogen peroxide was diluted with phosphate buffer pH 7.0 and its initial absorbance was adjusted between 0.5 to 0.6 absorbance units at 240 nm. The decrease in the absorbance was measured. One unit of catalase activity was defined as the amount of catalase which absorbed in 30 s at 25 °C. The catalase activity was then calculated from the change in absorbance and finally expressed as U ml^{-1}.

Total Antioxidant Capacity (TAC) Measurement Plasma total antioxidant capacity was determined by the ferric reducing ability of plasma (FRAP) assay according to the procedure of Benzie and Strain [22]. FRAP assay is based on the principle of reduction of ferrictripyridyltriazine (Fe3+-TPTZ) complex to ferrous tripyridyltriazine (Fe2+-TPTZ) by the antioxidants of a sample at low pH. The FRAP assay, a single electron transfer reaction, was conducted as previously described [30,36,38]. The FRAP assay utilizes water-soluble antioxidants native to the plasma collected from EDTA-treated blood to reduce ferric iron to the ferrous form subsequently producing a chromogen identifiable at 593 nm (Synergy H1Hybrid Reader, BioTek Instruments Inc., Winooski, VT, USA. The ferric-reducing antioxidant power value of the samples tested is expressed as an equivalent of the concentration of a

water-soluble vitamin E-analog, Trolox solution.

STATISTICAL ANALYSIS

All results were expressed as mean ±SD and differences were considered one-way ANOVA followed by post hoc LSD test to evaluate differences between different groups. P < 0.05 was considered significant [SPSS software (Statistical Package for the Social Sciences, version 19.0, SPSS Inc, Chicago, Illinois, USA].

RESULTS

In this cross-sectional study among the patients thirty four patients were enrolled. Data on age, Sex, Dialysis duration, Dialysis time per week are given in Table 1. The average age of the participants was (55 ± 16) years, 52.9% were female. The average time of dialysis treatment was equal to 29 ± 16 months. However, the average dialysis time per week was 10.1 ± 1.8 h. *All patients* were on *maintenance hemodialysis* and their blood samples were taken before, during and after dialysis session. Baseline clinical and biochemical characteristics of patients are given in Table 1 too. The average level of Residual creatinine clearance, Iron and Blood sugar respectively were 9.01 ± 0.6 (mg dl^{-1}), 120.30 (mg dl^{-1}) and 104.06 ± 14.6 (mg dl^{-1}). In Table 1, the average level of Blood urea, Triglyceride and Albumin were 93.16 ± 9.15 (mg dl^{-1}), 198/75 ± 39.5 (mg dl^{-1}) and 4.19 ± 0.32 (g dl^{-1}), respectively. The blood samples were diluted with the appropriate diluting fluids for red blood corpuscle (RBC) and white blood corpuscle (WBC) counts and the counts were determined using an improved Neubauer hemocytometer and then calculated. The *Hematocrit*, RBC and WBC count in the patients respectively were 34.14 ± 4.08 (Hct)%, 6.5×10^3 ($10^6 \times$ µl) and 3.59×10^6 ($10^3 \times$ µl).

Table 2 shows the erythrocyte catalase activity of before, during and after HD patients. Erythrocyte catalase activity was significantly (P < 0.001) reduced in during (107.01 ± 36.2) and after (105.30 ± 36.75) HD patients as compared with before (159.75 ± 30.98) HD patients. Further, the total antioxidant level significantly (P < 0.001) elevated during (648.52 ± 14.66) and after (573.08 ± 15.16) dialysis in HD patients.

DISCUSSIONS

The aim of the present study was to evaluate oxidative stress biomarkers as measured by plasma levels of catalase and Total antioxidant capacity levels enzyme of before, during and after dialysis in HD patients. Globally, an estimated 387 million people, or 8.3% of the population, have diabetes according to the International Diabetes Federation (IDF) Diabetes Atlas update of 2014 [23]. Diabetes accounted for approximately 45% of patients with end-stage renal disease (ESRD) in the US Renal Data System in 201 [24,25]. Patients with end stage renal disease failure (ESRD) undergoing long term hemodialysis (HD). Haemodialysis is known to be one major causes of oxidative stress due to the activation of polymorphonuclear neutrophil leukocytes by contact with the dialysis membranes. Interaction of circulating blood with the hemodialytic system causes bio-incompatibility reactions, such as production of ROS by inflammatory cells due to complement-dependent or complement-independent pathways [26]. The majority of ROS generated from activated polymorphonuclear leukocytes (PMNL) is hydrogen peroxide that leads to subsequent lipid peroxidation. Activated PMNL have been reported earlier to mediate lipid peroxidation in red blood cells [27]. There are various reports on changes in plasma lipid peroxidation and erythrocyte antioxidant enzyme due to hemodialysis [28]. Scientific research evidenced decline in the number of leukocytes (neutrophils, lymphocytes and monocytes) after dialysis [29]. Hydrogen peroxide is a harmful byproduct of many normal metabolic processes; to prevent damage to cells and tissues, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules [30]. Blma *et al.* reported that the activities of erythrocyte antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase got significantly decreased following hemodialysis [31]. It has been suggested that a decrease in the activities of primary antioxidant; CAT, may be due accumulation of ROS [32]. The inhibition of antioxidant system may lead to accumulation of H_2O_2 or products of its decomposition may also be aided by a decrease in CAT

Table 1. Clinical and Biochemical Characteristics of 34 Patients on HD

No.	Parameter	Mean ± SD
1	Age (y)	55 ± 16
2	Sex (Male/Female)	16/18
3	Dialysis duration (M)	29 ± 16
4	Dialysate buffer	Bicarbonate
5	Dialysis time per week (h)	10.1 ± 1.8
6	Residual creatinine clearance (mg dl^{-1})	9.01 ± 0.6
7	Iron (mg dl^{-1})	120.30
8	Hb (mg dl^{-1})	11 ± 1.3
9	WBC ($10^3 \times$ μl)	6.5×10^3
10	RBC ($10^6 \times$ μl)	3.59×10^6
11	Blood sugar level (mg dl^{-1})	104.06 ± 14.6
12	*Hematocrit* (Hct)%	34.14 ± 4.08
13	Blood urea (mg dl^{-1})	93.16 ± 9.15
14	Triglyceride (mg dl^{-1})	198/75 ± 39.5
15	Albumin (g dl^{-1})	4.19 ± 0.32

Data are expressed as mean ± SD.

Table 2. Levels of Antioxidant Markers in HD Patients

Antioxidant markers	Before HD	During HD	After HD
CAT (U ml^{-1})	(159.75 ± 30.98)	(107.01 ±36.2)	(105.30 ± 36.75)
TAC (μmol Trolox l^{-1})	(486.61 ± 14.86)	(648.52 ± 14.66)	(573.08 ± 15.16)

(CAT) catalase; (TAC) total antioxidant capacity. Data are presented as the mean ± standard division. Statistically significant between all the groups ($P < 0.001$).

activity [33]. Measurement of this antioxidant enzyme is an appropriate way to assess the pro oxidant antioxidant status [34]. We have also observed significant decrease in the erythrocyte catalse activity after hemodyalysis. Rico *et al.* have obtained decreased erythrocyte catalase activity in post dialysis when compared with pre dialysis and control group [35]. Stępniewska *et al.* also reported similar trend of

catalase enzyme activity [36]. The results of this study showed that catalase activity during and after dialysis compared to before dialysis is reduced. Likewise, a decrease in catalase, a key enzyme for the detoxification of hydrogen peroxide and organic hydroperoxide, accompanied by increased nitric oxide (NO) inactivation and protein nitration by reactive oxygen species (ROS) [37], has been

linked to the pathogenesis of chronic kidney diseases related hypertension via a dysregulation of NO function.

Total antioxidant capacity is an indicator of plasma resistance against oxidant agents. The major advantage of this test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound. The major contributors to the total antioxidant capacity of plasma are urate, ascorbate, vitamin E, and plasma protein [38]. We have measured this status in the form of ferric reducing ability of plasma (FRAP). A significant increase in during and after dialysis in FRAP levels were observed in our study, which is in accordance with earlier reports [39]. Another clinical study reported that the process of blood contact with dialysis membrane could have stimulated production of reactive oxygen species. Several studies have suggested that the presence of inflammation and the duration of dialysis are the most important determinants of oxidative stress in HD patients [40]. It is essential to maintain control over free radicals and antioxidants in order to maintain normal physiological homeostasis.

CONCLUSIONS

HD patients faced marked disturbances in the antioxidant metabolism have been reported by various authors which is exceeding by a dialysis procedure. In HD session, when conducted with a biocompatible membrane, appears to play an important role in the elevated intracellular ROS in patients and imbalance between ROS production and antioxidant defence. Therefore, increased oxidative stress may play an important role in the therapeutic target for pharmacologic therapy and palliation of hemoincompatibility of a dialysis system patients undergoing HD.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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