Research Article

Effect of Hemodialysis & Dialyzer Biocompatibility on Erythrocyte Glutathione &Related Enzymes on Uremic Patients

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Abstract

Twenty five healthy control and forty two end stage renal disease (ESRD) patients on regular hemodialysis treatment were enrolled in this study. Blood samples Were drown immediately before and after hemodialysis, and erythrocyte glutathione peroxidase (GSH-Px), glutathione reductase (GSSG-RD) activities, as well as reduced glutathione (GSH) concentration were measured. To study the effect of a single hemodialysis session on glutathione defense system and oxidative stress, the patients were then divided into two groups according to the dialyzer type used in the session (cuprophane, n=23 patients and polysulfone, n=19 patients).

GSH-Px activity, as well as GSH concentration was significantly decreased in ESRD patients as compared with controls. GSSG-RD activity was significantly elevated in ESRD patients as compared with controls. A single hemodialysis session, regardless to the type of dialyzer used, did not induce any significant effect on any of the parameters measured. Cuprophane dialyzer did not result in any significant changes among glutathione defense system parameters. Polysulfone dialyzer exerted a significant correction on glutathione system parameters. The findings conclude that the Glutathione defense system may serve as a good index for monitoring oxidative stress and dialyzers biocompatibility in ESRD patients on regular hemodialysis treatment and the use of polysulfone rather than cuprophane dialyzers in hemodialysis procedure is recommended.

Keywords: recurrent heel pain syndrome, planter fasciitis, calcaneal spur excision.

INTRODUCTION

Reactive oxygen species (ROS) is a 'term that encompasses all highly reactive, oxygen-containing molecules, including free radicals. Free radicals are evolved in the using of oxygen during normal metabolism within the cell to create energy (oxidation) [(1)]. They essentially have an electrical charge and desire to get and electron from any molecule or substance in the vicinity. ROS have numerous deleterious effects on cells, including lipid peroxidation [(2)], oxidation of all proteins and damage to DNA [(4)] .Fortunately, ROS formation is controlled by various beneficial compounds known as antioxidants. Among these antioxidants the glutathione defense system represents one of the most powerful machinery in competing with oxidative radicals and critically electrophilic toxins [(2)]. Glutathione defense system compressed of reduced glutathione (GSH), glutathione peroxidase (GSH-Px), and glutathione reductase (GSSG-Rd). Reduced GSH is used by GSH-Px to detoxify hydrogen peroxide and organic peroxides to water and organic alcohol, respectively, with the liberation of oxidized GSSG (glutathione disulfide). GSSG then is recycled under the action of GSSG-Rd enzyme and the reaction requires NADPH provided from the enzyme elucose - 6- phosphate dehydrogenase (G6PD)[(6)].When there is an increase in ROS production and/or decrease in the antioxidative mechanisms a state named as "oxidative

stress" is established [7]. Oxidative stress has been proposed to play a role in many states often associated with end-stage renal diseases (ESRD), including cardiovascular and infectious diseases, cancer, diabetes, disorder of peripheral and central nervous system, anemia, and accelerated aging (1, [9.11°]). Several hypothesis have been constructed to explain the factor implicating oxidative stress in ESRD patients especially those who had regular hemodialysis treatment. Among these factors are continuous loss of important water soluble antioxidants such as vitamin C to the dialysate("); The dialysate buffer[12],iron supplementation which could arise ROS generationby catalyzing fentor reaction[13] and the absence of complete correction of uremia toxicity and malnutrition[14].However, the interaction between blood component and dialyzer memberane has been considered among broad number of investigators as one of the most important factors established and submitted to an extensive studies [15,16,17.19).

Passage of blood through the dialyzer alters the physiology of WBC,especially the granulocyte fraction with the consequence of ROS release[20]. The interaction between blood components and dialysis membrane is governed by dialyzer biocompatibility. When we say we have a high biocompatible dialyzer we mean that it has the lowest ability to activate and interact with blood cells. Most comercial dialyzers are of two types: Modified biologic materials (such as cuprophane, hemophane, cellolus triacteate , ... etc), and synthetic materials (such as polysulfone, polycarbonate, polyacrylonitrile, ... etc) [21].

In general synthetic material are more biocompatible than the counterpart the modified biologic materials and many of the adverse pathobiologic consequences of hemodialysis that arise from membrane interactions are absent or attenuated by dialysis against synthetic membrane materials. In contrast, hemodialysis with cellulosic membranes is associated with the greatest number of acute events and perhaps long term complications [22,23]. Because of the frequent reports that hemodialysis patients are at high risk of oxidative stress, the imbalance between the oxidants and antioxidant have been extensively investigated (about 100 citation sinc(1990) [24]. And considering dialyzer incompatibility as the major contributor to the hightened oxidative state in hemodialysis patients, new approaches to the antioxidant therapy in hemodialysis have been interacted in recent years, namely hemolipodialysis, infusion of antioidaints by dialysate, and vitamin E-bonded membrances [14]. Adverse results have been obtained among broad number of investigators from different citations regarding the status of glutathione antioxidant system in ESRD patients. For each member of the system (GS11, GSIIPx, or GSSG-Rd), the results were rangine from increased, normal, or *decreased* levels in erythrocytes of uremic subjects (those conflecting reports will be pointed to in the following discussion). These controversies are even extended to include the results from measuring glutathione- defense system before or after dialysis , in a single session ,within session , or after time duration. As suggested by Gaetani et al [25],those contradictions might be derived from the heterogenity of ESRD patients samples and laboratory protocols.

In a pioneer study, we aimed to elucidate the effect of two different dialyzers in their biocompatibility (polysulfone & cuprophane) on erythrocyte glutathione defense system in ESRD patients on regular hemodialysis treatment, and also to determine if glutathione defense system *can* serve as a discrimenatory index to differentiate between these two standard commercial dialyzers.

PATIENTS AND METHODS

A total of 42 patients (28 Males , 14 Females) of various etiologies were recruited from the artificial kidney units in Al-Yarmuk (n=20 patients), and AL-Kadhemia Teaching Hospitals(n=22 patients). The patients ages were ranging from(18-67) years (mean ± SD 42.2 ± 13.9 years). Those patients with acute infection, chronic inflamatory disease, respiratory diseases, hepatic disease, and recent history of blood transfusion were excluded. The diet of the dialysis patients was not modified from that already prescribed for their end-stage renal disease.

The patients received dialysis for 3-4 hours in each session , 2 times weekly with the aid of AltraTouch 1000 equipment (Darke Willowk Sweden), using acetate as buffer.To study the effect of a single dialysis session with two different dialyzers on patients variables all patients (n=42 pts.)were subdivided to two categories' those who had a single dialysis session using low- flux cuprophane (belle., model E2, Italy) dialyzers (n=23 pts), and those who had dialysis using low- flux polysulfone (Fresenius, model F6HPS, Germany) dialyzers (n=l9pts).Twenty five healthy volunteers of age and sex match were served as controls. None of the controls was a heavy smoker, alcoholic, on any special diet, taking any antioxidants or had any history of rend problems before taking part in this study. Five milliliters of blood were drown from the arteriovenous fistula immediately before hemodialysis (pre —HD) and after hemodialysis (post — HD). Blood

samples were anticoagulated with sodium citrate and centerfuged at 700g for 10min. Plasma and buffy-coat were then removed by aspiration. The erythrocytes were washed three times with phosphate buffered saline (PH 7.4; 0.02 M Phosphate; 0,123 M NaC1). The packed erythrocyte volume (PCV) after the final wash was used to determine the activities of the enzymes GSH-Px and GSSG-Rd as well as GSI-1 concentration.

Glutatione was assayed according to the procedure of Beutler [(26)] with some modification from Tietz[(27)]. GSH —Px activity was assumed *according* to the procedure of Paglia & Valantine [(28)] with slight modifications from Hopkins &Tudhope [(29)] and also from plemban et al [(30)]. GSSG-Rd[(31)] activity was determined by the method of west et at al[(31)] with minor modifications from Lee etal **[(32)]**..

RESULT

Table (1) represents the levels and significancy of erythrocyte GSH, GSH-Ps and GSSG-Rd among healthy individuals and ESRD locals (Pre-HD and Post —HD). The mean (±SD) value of GSH in

pre –Hd) patients was 5.55 ± 1.21 µmol/gHb (range 3.89-7.86 pnioligHb), ,which was significantly lower than that of normal controls by —25% (p<0.0001). The level GSH after dialysis (post —HD) was slightly but not significantly increased with a mean (±SD) of 6.04 ± 1.16µmol/gHb, and this was significantly lower than that of control (p<0.0001). GSH-Ps activity for ESRD patients (Pre-HD) had also shown a significant decrease by —31% (P< 0.001) as compared with control group. Dialysis procedure did not result in any change in enzyme activity, and GSH-Ps activity after dialysis remained significantly below that of controls (p<0.0001). On the other hand erythrocyte GSSG-Rd activity for ESRD patients had revealed a marked elevation with mean (±SD) of 9.31 ± 2.08 U/gHb as compared with controls mean value [6.85± 0.89 1.//gHb (p<0.0001)]. After singnificantly elevated as compared with control mean of activity (P<0.001), but not different as compared with that of pre —HD group.

Table (2) indicates the effect of polysulfone and cuprophone membranes of erythrocyte GSH concentration. When ESRD patients had dialysis with cuproplane membranes no significant change was observed in GSH level between pre-HD and post —HD groups (5.77±1.14 µmol/gHb,pre-HD VS 5.67±1.08 µmo/gHb, post-HD). On contrast, polysulfon membranes caused 8.9% increase in GSH level from 5.94±1.33 µmol/gHb in pre-HD to 6.47±1.12 µmol/gHb in post –HD with P<0.01. the same effect was observed for polsulfone membrane on erythrocyte GSH-Px activity as indicated in table (3). A correction of 8.37% from 20.3+ 2.82 U/gHb in Pre- HD group to 22.0± 2.48 U/gHb in post group in GSH-PX activity was seen after polysulfone dialysis(P<0.0001), while cuprophane membrane exerted a nigligable change in enzyme activity (table 3).

However, although polysulfone dialyzers have increased GSH and GSH- PX levels, the mean values of both two parameters remained significantly below that of normal control (tables 2 and 3 resectively). GSSG-Rd activity was also affected notably by polysulfone dialyzers, but in a reverse manner, the activity of erythrocyte GSSG-Rd was declined significantly after polysulfone dialysis from 9.23± 2.31 U/gHb in pre-HL) group to 8.78± 2.29 U/gHb in post-HD group with p>0.05 (table 4), while cuprophane dialysis did not lead to nay change in enzyme activity. Like GSH and GSH-PX, the rebound in GSSG-Rd level after polysulfone dialysis was not enough to establish controls level and it remained significantly elevated (p<0.01, table 4).

Tabte.1 Comparison of GSH, GSH-Rd activities in control and ESRD patients (pre-and post- dialysis

parameter	groups	Number of cases	Level* mean ±SD	range	significantly	% difference
GSH	Controls Pre-HD Post -HD	25 42 42	7.79±0.75 5.85± 1.21 6.04 ±1.16	5.25-9.29 3.89-7.86 3.93-8.90	P<0.0001a.b NS c	-25%a, -22.4%b +3.24%c
GSH-Px	Controls Pre-HD Post -HD	25 42 42	28.81±1.92 19.9 ±2.29 20.5±2.50	22.3-35.5 16.4-27.0 15.9-26.6	P< 0.001 a,b NS c	-31%a,-28,8%b +3%c
GSSG-Rd	Controls Pre-HD Post -HD	25 42 42	6.85 ± 0.90 9.31±2.08 9.15±2.18	5.63-8.50 5.75-13.3 5.31-13.3	P<0.001 a,b NS c	+36%a,+33 5%b -1.72%c

***** The units for GSH concentration is µmol/g.lb, and for GSH-Px and for GSSG-Rd activities are U/g1-11).

a- Control VS pre-HD

b- Control VS post-I ID

c- Pre-HD Vs post-HD. NS: Not Significant

Table 2. Comparison of GSH levels in Control and ESRD patients (pre-and post-dialysis] according to dialyzer type

GSH (μ mol/gHb)	Control	Cuprophane		Polysulfone	
		Pre-HD	Post-HD Pre-HD		
N	25	23	23	19	19
Mean ± SD	7.79	5.77	5.67	5.94	6.47
	10.75	±1.14	±1.08	±1.33	±1.12
%:(C VS pre)		-26%		-23.7%	
(C VS post)			-27.2%		-17%
Pre VSost			1.7%		+8.9%
P:(C VS post)			<0.01		<0.01
(Pre VS post)			NS <0.01		<0.01

Table 3. Comparison of GSH-Px activities in Control and ESRD Patients (pre - and post-dialysis) according to dialyzer type**.**

GSSG-Rd (U/g Hb)	Control (C)	Cuprophane		Polysulfone	
		Pre -HD	POST -HD	Pre -HD	Post -HD
n	25	23	23	19	19
Mean ±SD	6.85 ±0.90	9.85 ±1.93	9.45 ±2.31%	9.23 ±2.31	8.78 ±2.29
%: (C VS pre) (C VS post) (pre VS post)		+37%	+38% 0.74%	+34.7%	+28% -4.87%
P: (C VS post) (pre VS post			<0.01 NS		<0.01 <0.05

Table 4. Comparison of GSSG-Rd activities in control and ESRD Patients (pre – and post- dialysis) according to dialyzer type

GSH-Px (U/gHb)	Control	Cuprophane		Polysulfone	
		Pre-HD Post-HD		Pre-HD 1	Post-HD
N	25	23	23	19	19
Mean ± SD	28.8 ±1.92	19.6 ±1.73	19.4 ±1.92	20.3 ±2.82	22.0 ±2.48
%:(C VS pre) (C VS post) (Pre VS post)		- 32%	-32% - 1%	-29.5%	- 23.6% +8.37%
P:(C VS post) (Pre VS post)			<0.0001 NS		<0.0001 <0.0001

DISCUSSION

For erythrocyte GSH level, previous reports have had conflicting *findings* of low [33,34,36,37,38,39,40,41],normal [42,43,44,45], or high [25,49] levels. Ross et at [1401] who measured GSH with highly validated HPLC method suggested that those discrepancies might related to *differences* in either assay methodology or subject characteristics.In this study, the mean level of erythrocyte GSH in ESRD patients was 5.85 jumoligHb which is 25% lower that of healthy subject (Table 1). This decrease might be derived from the continuous consumption of erythrocyte reduced glutathione in removing oxidative substances and electrophilic toxins that could accumulate due to absent renal clearance also the loss of essential water soluble vitamins such as folic acid and pyrodoxine in regular dialysis treatment [50]could contribute to reduce glutathione for scavenging oxidants. It has been found that glutathione synthesis is increased upon supplementation with these vitamins ([51]). Furthermore, the loss of vitamin C during dialysis treatment and absence of exogenous supplementation [11] might overburden glutathione antioxidative function and accelerate its turnover. Jacob [57] has suggested that glutathion and Vitamin C work interactivity to quench free radicals and that they have a sparing effect on each other.

Erythrocyte GSH-Px level had also revealed a significant decrease in this study by 31% for ESRD patients as compared with controls (Table 1). This result "as in agreement with previous records [34,36,53,54,56,57,37,58,38,59,60,61] and in contrast with other who found normal [62]or high [39] enzyme activity in uremic patients.many factors might contribute to lower GSH-Ps activity in ESRD patients. Circulating uremic inhibiter could share a part in GSH-Px inactivation. Richard et al (53) suggested the involvement of an endogeneous tonic ligands and proposed indolacetic acid, indoxysulfate, 3,4- carboxy-5-methyl-2-propyl furancarboxylic acid, and MDA as potential candidates. Furthermore, it is possible that post-translation modification of the enzyme could contribute to impair its activity. A number of modicications are possible, including glycation, oxidation, and carbamylation. GSH-Px is reported to be inactivated by advanced glycation end-products in vitro [63]. Advanced glycation end-products are increased in uremia [64]. So, GSH-Px activity could be reduced due to inactivation by glycation. Isocynate which is formed from the spotaneous decomposition of urea [65] may also inhibit the enzyme by carbamylation. Significant carbomylation of several proteins has been shown to occur in uremic subjects[66,67]. Roxborough et al[68] has demonstrated that minimal carbamylation of GSH-Px significantly reduces enzyme activity in vitro.Unlike to GSH and GSH-Px, erythrocyte GSSG-Rd activity of ESRD patients was 36% higher than that of control (Table 1). This is inaccordance with Melissions et al [9]; Suzuki etal 309; Pasaogln et al [38]; and Cetallos et al, and in contrast with El-Rashidy et at [16] who found normal GSSG-Rd activity, and Hassan et al who found low activity six uremic patients. The augmentation of GSSG-Rd activity could be explained by the elevation in the oxidized form of glutathions (GSSG) in plasma (' and erythrocyte [71] as well as mixed —disulfides between glutathione and Haemoglobine or other protein free sulfohydryl groups (., which are known to be substrate for GSSG-Rd [73]. Accordingly, the alteration in enzyme activity could be an adaptive response to compensate with the elevation of these substances and other oxidizing waste products.A single Hemodialysis session did not result in any significant effect an glutathione defense system parameters(Table.l). Previous reports have measured the effect of a single dialysis session on each of the glutathione system parameters individualy and found the same results [74'61].The only report measured the effect of hemodialysis session of glutathione system as one unit is that of Hassan et al (5s). He found that a single dialysis session exacerbate the impairment in erythrocyte glutathione system. We believe that the results illustrating the effect of hemodialysis on the antioxidant system would be obscure unless otherwise verfied with the type of dialyzer used in hemodialysis procedure. In this was the case where we divided the patients according to the dialyzer type (cuprophane and polysulfone). Those patients who had dialysis with cuprophane membrane did not show any change in the levels of GSH, GSH-Px, and GSSG-Rd, while polysulfone membranes caused a significant enhancement in the levels of GSH and GSH-Px as illustrated in tables [2] and [3], respectively and also a significant rebound in the activity of GSSG-Rd as illustrated in table (4). To our knowledge, this is the pioneer study in which the effect of these two commercial dialyzers on erythrocyte glutathione defence system is determined.Previous investigations used other types of dialyzers such as polyacrylonotrile, polymethyl methacrylate, polycarbonate, hemophane, … etc. [17,15,75&76].the result obtained from this study indicate the beneficial effect of polysufone dialyzers on erythrocyte glutathione system in ERRD patient.The improvement in glutathione system upon polysulfone dialysis may be ascribed to the partial removed of high meolecular weight toxins that have an oxidant activity which overborden

erythrocyte glutathione system. polysulfone membrane is known to be more effective

in the clearance of high molecular weight toxins (up to 10.000 Daltons or more) than cuprophane membrane (< 300 Daltons) [21]. Moreover, Roselaar et al[77] has detected potent oxidants that have an upper molecular weight limited of about 3,000 Daltons. He also found that these oxidants are dialyzable from uremic plasma. Consequently, it could be the removed of these oxidants that had led to the partial correction in glutathione system parameters that we observed upon polysulfone dialysis. Furthermore, the higher biocompatibility of polysulfone dialyzers with their lower potency to activate blood leukocyte and hence lesser degree of ROS release may also participate in improving glutathione defence system.

In conclusion, we believe that it would more convenient to use polysulfone rather than cuprophane as the dialyzer of choice in the treatment of ESRD patients with hemodialysis strategy. It is also noteworthy for those researchers who work in the field of clinical nephroloey to take in their account the effect of dialyzers type when submitting a sampling protocols, since dialyzers are no more se-en as a simple semi-permeable barriers for solutes and water, but is now considered as an important interface with the patient·s blood, and subsequently, as an outcome predicators.

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