The impact of hemodialysis session on red cells antioxidant enzymes (Glutathione Peroxidase and Reductase) in end stage renal disease patients undergoing maintenance hemodialysis: Controlled trial

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ABSTRACT

Objective: Anemia is common in (End stage renal disease) ESRD patients. Oxidative stress exacerbate anemia. we aim to assess the impact of hemodialysis (HD) procedure on red cell antioxidant enzymes.

Methods: A controlled trial involving 30 HD patients and 30 controls; blood samples were taken pre- and post-HD for assessment of red cell glutathione peroxidase (GSH-Px) and reductase (GSH-Rx).

Results: Post-HD; GSH-Px, GSH-Rx were significantly reduced compared to both Pre-HD and control group **(**P<0.001) respectively. Patients on erythropoietin and iron therapy had significant higher GSH-Px enzyme compared to patients on iron therapy GSH-Px had significant inverse correlation versus serum creatinine and urea and positive correlation with Hct and total protein. Regression analysis showed that HD therapy duration (P<0.001), BMI (*P* = 0.004), serum urea, creatinine, Hct, and total proteins (P<0.001) are significant factors affecting red cell GSH-Px; whereas serum creatinine, urea, neutrophils count, total proteins (P<0.001) and red cell parameter MCV (*P* = 0.002) and Hct (*P* = 0.04) are significant factors affecting red cell GSH-Rx. Receiver operator characteristic (ROC) curve for GSH-Px and GSH-Rx cut off point which discriminate between HD patients and normal control is (86) and (69) (nmol/min/ml) respectively with 100% sensitivity and specificity. Area under the curve is 1.0.

Conclusion: Mechanical stress during extracorporeal circulation in the dialysis machine aggravates red cell oxidative damage. Therefore it is suggested to supplement erythropoietin together with antioxidant as they may be capable of recovering antioxidant defense in HD patients.

KEY WORDS: RBCs glutathione, Oxidative Stress, Hemodialysis, ESRD

Introduction

The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress" (OS). Regulation of a balance of antioxidant and OS is critical for cell viability, activation, proliferation and organ function. Reactive oxygen species (ROS) are produced by living organisms during normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are harmful molecules; which can damage cell structures and functions. Aerobic organisms have integrated antioxidant systems, which include both enzymatic and non-enzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, in pathological conditions, the antioxidant systems can be disturbed [1].

OS has a critical role in the pathophysiology of several kidney diseases and many complications of these diseases are mediated by OS or by OS-related mediators and inflammation [2], so one reason for OS in patients with renal failure is the underlying disease itself.

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However, treatment procedures such as hemodialysis (HD) can induce OS because of massive repeated contact of blood with dialysis membranes that triggers defense mechanisms in the red cells to protect against oxidative damage; together with chronic deficit in antioxidant defense system [3].

The red cells play a key role in maintenance of the systemic and local antioxidant defense system as they form the "first line of defense" during contact of the blood with the dialysis membrane. The effect of ROS on red cells appears in the form of reduced osmotic resistance of their cellular membrane and susceptibility to disintegration, hemolysis and significant decline in red cells lifespan [4]. Moreover; direct contact of the blood with the dialysis membrane provokes a chain of changes in blood cells; resulting in reduction of white blood cell count and lymphocyte number, stimulation of neutrophil de-granulated and increase in platelet adhesiveness. In addition; interactions of granulocytes with the dialysis membrane stimulate the production of ROS and activate aerobic reactions causing OS [5].

Anemia associated with chronic kidney disease (CKD) develops gradually during the progressive decline of renal function and it is characterized by a relative paucity of erythropoietin secretion from the diseased kidney [6]. Anemia is worsened in chronic renal failure due to blood loss from laboratory tests and during HD or gastrointestinal losses and poor appetite with reduced protein intake [7].

Anemia can augment OS by increasing tissue ROS generation during anaerobic metabolism and reducing antioxidant defense because of the diminished red cells pool [7, 8].

GSH-Px is the designated primary defense for removal of red cell reactive species; in addition; GSH-Rx plays a critical role by regenerating reduced glutathione from the oxidized form hence protects red blood cells against hemoglobin oxidation and hemolysis [9]. Therefore, evaluation of red cell GSH-Px and GSH-Rx is a reliable method for studying red cell antioxidant system. The study aimed to assess the impact of HD session on red cells antioxidant enzymes activities (GSH-Px and GSH-Rx) in ESRD patients on regular HD.

Materials and methods

Study design and subjects

A clinical trial involves 30 ESRD patients on maintenance HD (23 males and 7 females) attending the nephrology unit of Naga Hamadi general hospital, Upper Egypt; who agreed to enter the study. Thirty healthy volunteer (21 males and 9 females); free fromkidney disease and with normal hematological and biochemical values, and twere not receiving any medication known to interfere with the studied variables served as control. The study conducted after approval of Research Ethics Committee in Qena Faculty of medicine and after taking an informed consent from the patients and subjects who participated in the present study.

Exclusion criteria

Patients with autoimmune disease, pregnancy, malignancy, hematological disorders, infections, HBV or HCV and HIV positivity, hyper parathyroidism, blood loss or transfusion and known cause of anemia (e.g., haemoglobinopathies) were excluded.

Inclusion Criteria

All patients attended the dialysis unit 3 times per week; each dialysis session lasted for 4 hours, using bicarbonate dialysate solution with polysulphone F7/F9 membrane dialyzers (Bio-140; Dialife SA, Taverne, Switzerland).

All patients' received medication including: anti-hypertensive drugs (calcium antagonists, angiotensin converting enzyme inhibitors), phosphate binders (calcium carbonate), those receive recombinant erythropoietin (EPO) therapy by the subcutaneous route together with parenteral iron and those receive iron only by intramuscular injection by the end of HD. The following characteristics were observed or calculated: age, gender, duration of dialysis, weight, height, and body-mass index (BMI). Other relevant dialysis data included the cause of renal failure, duration of dialysis, and presence of co-morbidity (e.g. diabetes mellitus, hypertension and arthrosclerosis).

Blood samples

7 ml venous blood samples were taken twice from HD patients; pre-HD and post-HD on a midweek dialysis sessions. The pre-HD sample was drawn from arteriovenous fistulas at insertion of the arterial needle, before heparinization of the line and after an overnight fasting period and before the patient was being connected to the dialysis machine and the post-HD sample just prior to patient disconnection and blood samples were taken once from the control subjects. Blood samples were collected in EDTA (2ml), heparin (2ml) and plain (3ml) vacutainer tubes. EDTA tube were used for complete blood cell count (CBC) using cell dyne-1800 (Abbott diagnostics, Santa Clara; California-USA). Plain tube bloods were allowed to clot and then centrifuged at 3000 rpm for 10 minute and serum separated for colorimetric assessment blood chemistry using Cobas c311 automated analyzer (Roche diagnostics, Mannheim- Germany). For heparinized tube all operations performed at 4 °C; first sample centrifuged at low speed (700 g) for 10 minutes. Plasma and buffy coats were carefully aspirated from the surface of the pellet. The remaining red blood cells were washed three times with isotonic saline, centrifuged as described above and after the final wash were re-suspended in an equal volume of isotonic saline and then red cell hemolysate prepared aliquoted in 1 ml cryo-tubes and stored at -80 °C for later analyses of GSH-Px and GSH-Rx according to Andersen and his coworkers [10], and Esworthy et al., 2001 [11].

Red cell GSH-Px activity was measured indirectly by a coupled enzyme reaction with GSH-Rx measurements performed according to manufacturer protocol of kits provided by Cayman Chemical Michigan-USA; cat no 703102 and 703202 respectively. Oxidized glutathione (GSSG), produced upon reduction of hydro-peroxide by GSH-Px; is recycled to its reduced state by GSH-Rx and NADPH and the oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm at 37°C using the Humalyzer 2000 analyzer. A standard curve was prepared by using the standard provided in the kit and the value for each sample was read from the curve. GSH-Px activity precision: the intra-assay coefficient of variation was 5.7%. GSH-Px assay range 50-344 nmol/min/ml and the GSH-Rx activity intra-assay coefficient of variation was 3.7%; GSH-Rx assay range 20-255 nmol/min/ml. Calculations were done for body mass index (BMI): weight in kilograms divided by height in meter square (kg/m^2), Calculation of estimated Glomerular Filtration Rate (eGFR) (ml/min) using Cockcroft-Gault Equation as follows: Male = 140-age (year) X body weight (kg)/ 72 x serum creatinine (mg/dl) and for Females = 0.85 X [140-age (year) x body weight (kg) ÷ 72 x serum creatinine (mg/dl)] [12], and calculation of the percentage blood urea nitrogen reduction ratio (URR %) to assess the intensity of dialysis; inadequate dialysis (defined as a reduction in URR % of less than 65 % after a dialysis session) [13].

Statistical Analysis

Data analysis was performed using SPSS version 22 software. For quantitative data paired-sample and independent-sample two tailed Student *t* tests were used and Chi-Square was applied for qualitative data. Data are expressed in mean, standard deviation for quantitative data, numbers and percentage for qualitative data. Pearson correlation coefficient was used to explore the relationship between quantitative variables. A P value of less than 0.05 or <.001was considered significant. Linear regression was used to find out the effect of independent variables. Receiver operator characteristic (ROC) curve was performed to obtain the cut off points of red cell GSH-Px and GSH-Rx with sensitivity 100% and specificity 100% and area under the curve is 1.0.

Results

Baseline demographic characteristics and clinical data of the studied HD patients showed in Table 1. Laboratory findings shows statistically significant difference between pre- and post-HD groups compared to controls regarding urea, creatinine, uric acid, eGFR, total proteins, albumin, red cell indexes and red cell GSH-Px & GSH-Rx. Post-HD

Table 1. Demographic and clinical data of the HD patients.

Parameter (Mean ± SD)	ESRD Patients
Age (years) mean ± SD	61.9±9.99
Range :	44-81
Median:	59
Male : Female	23: 7
Duration of HD (months) mean ± SD	48.63±12.23
Range :	30-84
Median:	47.5
BMI (kg/m2) mean ± SD	22.15±6.32
Range :	14.85-35.2
Median:	23.3
Blood urea nitrogen reduction ratio (URR %) mean ± SD	60.18±9.35
Range :	37.5-77.5
Median:	61.275
< 65 %URR: No (%)	19(63.33%)
> 65 %URR: No (%)	11(36.66%)
Cause of ESRD:	
• **Chronic glomerulonephritis**	11 (36.7%)
• **Hypertension**	9 (30%)
• **Polycystic kidney**	1 (3.33%)
• **Systemic lupus erythematosus**	3 (10%)
• **Unknown**	6 (20%)
Co-morbidity:	
• **Diabetes**	15 (50%)
• **Hypertension**	11 (36.7%)
• **Atherosclerosis**	4 (13.3%)
Iron therapy:	
• **Parenteral iron**	22 (73.3%)
• **Combined Epo + Parenteral iron**	6 (20%)

BMI: body mass index; URR: urea nitrogen reduction ratio; Epo: erythropoietin

compared to pre-HD show statistically significant reduction in serum urea, creatinine, Hct, GSH-Px and GSH-Rx with significant increase in MCV (Table 2).

Pearson correlation shows statistically significant inverse correlation between GSH-Px versus serum creatinine, urea and BMI, and positive correlation versus Hct, total Protein and duration of HD therapy; otherwise no significant correlation versus other variables (Table 3a).

Pearson correlation shows statistically significant positive correlation between GSH-Rx versus MCV; otherwise no significant correlation versus other variables (Table 3b).

Patients who received EPO therapy with iron therapy have significant higher red cell GSH-Px enzyme activity compared to patients receiving iron therapy alone (Table 4).

Multivariate regression analysis showed that HD therapy duration (66%, $p < 0.001$), BMI (63%, $p = 0.004$), serum urea (55%, $p < 0.001$), serum creatinine (67%, $p < 0.001$), Hct (52%, $p < 0.001$), and total proteins (48%, $p < 0.001$) are significant factors affecting red cell GSH-Px level (Table 5).

Multivariate regression analysis showed that serum creatinine (30%, $p < 0.001$), urea (47%, $p < 0.001$), neutrophils count (32%, $p < 0.001$), total proteins (44%, $p < 0.001$) and red cell parameter MCV (42%, $p = 0.002$) and Hct (20%, $p = 0.04$) are significant factors affecting red cell GSH-Rx activity in ESRD patients (Table 6).

According to Lalkhen and McCluskey [14] validity parameters were calculated for red cell GSH-Px and GSH-Rx activity assessment in HD patients including: sensitivity [= ability to detect positive cases = true positive ÷ (true positive + false negative)]; specificity [= ability to exclude negative cases = true negative ÷ (negative + false positive)]; positive predictive value (PPV) = true positive /true positive + false positive = % of true positive cases to all positive; negative predictive value (NPV) = true negative ÷ (true negative + false negative) = % of the true negative to all negative cases and accuracy= (true positive + true negative) ÷ grand total. Moreover; Receiver operator characteristic (ROC) curve examining the values of red cell GSH-Px and GSH-Rx in HD patients showed that accuracy is measured by the area under the curve (AUC) of 1.0 ($P<0.001$). In this study; the optimal cut-off of red cell GSH-Px was (86 nmol/ min/ ml) and GSH-Rx was (69 nmol/ min/ml) which resulted in a high sensitivity of 100%, specificity of 100%, PPV of 100%, NPV of 100% and accuracy of 100% (Fig

Figure 1. ROC curve for red cell GSH-Px and GSH-Rx: The best cut off point which discriminate between HD patients and normal control is (86) and (69) (nmol/min/ml) respectively with sensitivity 100% and specificity 100%. Area under the curve is 1.0.

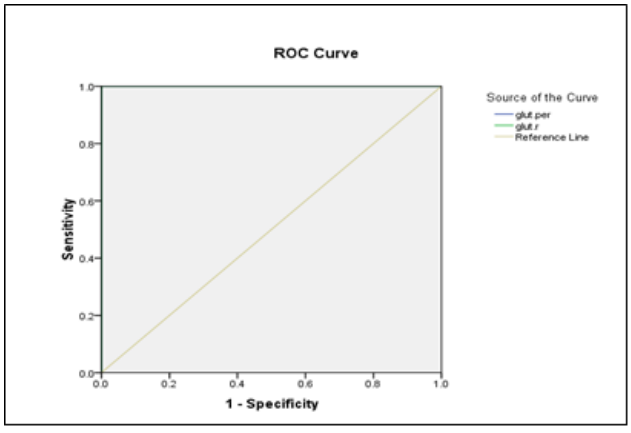

Table 2. Comparison of laboratory data between cases (pre- and post-HD) and with controls.

Parameter	**ESRD Patients**		**Controls**	***P value**
	Pre- HD	**post-HD**		
Urea (mg/dl) Mean ± SD	124±27	47.8±7	28±6	<0.001*[a]
Range :	88-177	35-66	20-37	0.004*[b]
Median:	113.5	47	27.5	<0.001*[c]
Creatinine (mg/dl)	10.9±1.5	5.9±1.1	1±0.4	<0.001*[a]
Mean ± SD	7.5-13.5	4.3-8.6	0.7-1.4	<0.001*[b]
Range :	11.1	5.65	1.05	<0.001*[c]
Median:				
eGFR (mL/min/1.73 m2)				
Mean ± SD	4.5±1	9.5±2	>60	<0.001*[a]
Range :	3-8	6-15		<0.001*[b]
Median:	4	9		<0.001*[c]
Uric acid (mg/dl)				
Mean ± SD	.5±3	8.6±0.6	4.4±2	<0.001*[a]
Range :	7.4-9.5	7.4-9.5	3.5-5.5	<0.001*[b]
Median:	8.45	8.45	4.45	0.23[c]
Total Protein (g/dl)				
Mean ± SD	5.9±0.7	5.9±1.1	7±0.6	<0.001*[a]
Range :	4.2-7.4	4.2-7.4	6.3-8	<0.001[b]
Median:	6	6	7	0.63[c]
Albumin (g/dl)				
Mean ± SD	3.6±1	3.7±1	4.3±1	<0.001*[a]
Range :	2.4-47	2.4-47	3.4-5.4	<0.001*[b]
Median:	3.6	3.6	4.5	0.35[c]
Hb (g/dl) Mean ± SD	8±2	7.6±1.3	12.7±2.3	<0.001*[a]
Range :	4.2-10.6	3.9-10.1	11.5-13.7	<0.001*[b]
Median:	8.35	8	12.7	0.11[c]
Hct (%)Mean ± SD	24.7±5	23±4	39.1±6	<0.001*[a]
Range :	14-34	13.4-31	36-45	<0.001*[b]
Median:	24.5	23.5	38	0.04*[c]
MCV (fl) Mean ± SD	78±5	80.7±11	86±5	<0.001*[a]
Range :	65-93.4	65-104	80-94	0.11[b]
Median:	75.95	79	86	0.02*[c]
MCH (pg) Mean ± SD	25±4.2	26.7±5	28±3.4	<0.001*[a]
Range :	20.7-32.5	22-37	25-33	0.23[b]
Median:	25	26	28	0.80[c]
MCHC (g/dl) Mean ± SD	32±3.2	33.4±3.6	32.7±5	0.001*[a]
Range :	27.2-40.2	28-44	31-37	0.31[b]
Median:	32.05	33.4	42	0.21[c]
RDW % Mean ± SD	13.9±2	13.9±2.1	12.9±2.5	0.03*[a]
Range :	10.1-19.1	10-19	11-16	0.27[b]
Median:	13.5	13.5	13	0.94[c]
Platelets count $\times 10^9$/L				
Mean ± SD	225±50	219±82	262±40	0.29[a]
Range :	107-475	100-470	188-351	0.49[b]
Median:	205	200	253	0.33[c]
WBCs $\times 10^9$/L **Mean ± SD**	7.5±3	7.5±1.3	7.9±3.1	0.80[a]
Range :	3.2-16.4	3-17	6.3-9.6	0.79[b]
Median:	7.4	7.2	7.9	0.96[c]
Red cell GSH-Px nmol/				
min/ml **Mean ± SD**	37.47±7.72	26.83±6.25	270.30±77.08	<0.001*[a]
Range :	22-48	15-36	124-432	<0.001*[b]
Median:	37.5	26	273	<0.001*[c]
Red cell GSH-Rx nmol/				
min/ml **Mean ± SD**	21±4.98	14.3±4.03	169.93±44.78	<0.001*[a]
Range :	14-35	9-22	103-298	<0.001*[b]
Median:	21	14.5	170	<0.001*[c]

* Unpaired t-test. ESRD: End stage renal disease, HD: Hemodialysis
Significant*****; a: pre-HD vs. controls; b post-HD vs. controls, c: pre-HD vs. post-HD

Table 3a. Correlation between Red cell GSH-Px versus anthropometric measures and laboratory parameters among ESRD cases.

Variables	Glutathione peroxidases			
	pre-HD		post-HD	
	r	P value	r	P value
HD therapy duration (months)	0.36	0.04*	0.411	0.024*
BMI (kg/m2)	-0.42	0.02*	-0.426	0.019*
Urea (mg/dl)	-0.56	<0.001*	-0.86	<0.001*
S. Creatinine (mg/dl)	-0.78	<0.001*	-0.88	<0.001*
eGFR (mL/min/1.73 m2)	0.01	0.92	0.12	0.62
Uric acid (mg/dl)	0.04	0.66	0.24	0.60
Hb (g/dl)	0.17	0.33	0.07	0.63
Hct (%)	0.76	<0.001*	0.73	<0.001*
MCV (fl)	0.20	0.34	0.25	0.39
Platelets count ×109/L	0.09	0.60	0.03	0.80
WBCs ×109/L	0.12	0.40	0.13	0.45
Total Protein (g/dl)	0.60	<0.001*	0.80	<0.001*
Albumin (g/dl)	0.29	0.13	0.27	0.13
Red cell GSH-Rx nmol/min/ml	-0.075	0.694	-0.012	0.95

Significant*

Table 3b. Correlation between Red cell GSH-Rx versus anthropometric measures and laboratory parameters among ESRD cases.

Variables	Glutathione reductase			
	pre-HD		post-HD	
	r	P value	r	P value
HD therapy duration (months)	0.0914	0.6309	0.1298	0.494
BMI (kg/m2)	0.0434	0.8199	0.1881	0.320
Urea (mg/dl)	0.0308	0.8717	-02331	0.215
S. Creatinine (mg/dl)	0.1414	0.4562	0.1933	0.306
eGFR (mL/min/1.73 m2)	-0.1161	0.5412	-0.198	0.294
Uric acid (mg/dl)	-0.1226	0.519	-0.053	0.782
Hb (g/dl)	0.2078	0.270	0.2726	0.273
Hct (%)	0.2349	0.211	0.2379	0.206
MCV (fl)	0.3641*	0.048*	0.2305	0.231
Platelets count ×109/L	-0.1679	0.375	-0.192	0.309
WBCs ×109/L	-0.0379	0.842	-0.107	0.574
Total Protein (g/dl)	0.1936	0.305	0.1512	0.425
Albumin (g/dl)	0.1362	0.473	0.1731	0.360
Red cell GSH-Px nmol/min/ml	-0.0754	0.692	-0.011	0.952

Significant* weak correlation

Table 4. Effect of iron and erythropoietin therapy on red cell GSH- activities.

Variables	Iron No=22	Epo + iron No= 6	t	P value *
Red cell GSH-Px mean ± SD Range : Median:	35.64±7.25 22-48 35.5	44.33±3.61 38-48 45	-2.81	0.0092*
Red cell GSH-Rx mean ± SD Range : Median:	21.59±5.36 14-35 21	19.83±3.54 15-23 21	0.754	0.4577NS

* Unpaired t-test. Epo: erythropoietin; Significant*; NS: non-significant

Table 5. Relation between red cell GSH-Px versus different variables by linear regression (multivariate analysis).

Variables	Beta-Coefficient	P value	R2
HD therapy duration (months)	0.67	<0.001*	0.66
BMI (kg/m2)	-0.51	0.004*	0.63
Urea (mg/dl)	-0.57	<0.001*	0.55
S. Creatinine (mg/dl)	-0.98	<0.001*	0.67
Hct (%)	0.46	<0.001*	0.52
Total proteins (g/dl)	0.40	<0.001*	0.48

Significant*; β standardized factor in the regression equation, R2 the coefficient of determination, P factor of significance,

Table 6. Relation between red cell GSH-Rx versus different variables by linear regression (multivariate analysis)

Variables	Beta-Coefficient	P value	R2
Urea (mg/dl)	-0.68	<0.001*	0.47
S. Creatinine (mg/dl)	-0.50	<0.001*	0.30
MCV (fl)	0.64	0.002	0.42
Hct (%)	0.30	0.04	0.20
Neutrophils %	0.44	<0.001*	0.32
Total proteins (g/dl)	0.20	<0.001*	0.44

Significant*; β standardized factor in the regression equation, R2 the coefficient of determination, p factor of significance,

Discussion

HD is an essential therapeutic approach for patients with ESRD. This procedure promotes a complex biological response when the patient's blood interacts with the synthetic HD membranes [15].

In the present study; All HD patients showed lower total proteins, albumin levels, hemoglobin, and eGFR ($p<0.001$), as well as higher urea, creatinine and uric acid levels ($p<0.001$) compared to healthy controls.

In this study; serum urea, creatinine and eGFR, showed statistically significant higher values in the pre-HD and post-HD groups compared to control group with significant decrease in post- HD compared to pre- HD values. The mean

URR% was 60.18±9.35 and mostly 19(63.33%) have inadequate dialysis with <65% URR% and 11(36.66%) have adequate dialysis with > 65% URR%.

In this study, patients with ESRD have significant reduction in red cell GSH-Px and GSH-Rx antioxidant enzyme activities in pre-HD compared to controls with further reduction in post-HD levels compared to pre-HD; this is in agreement with previous studies [16-21].

The decrease in the glutathione level in patients on HD may occur as a result of the inhibition of glutathione production, an increase in glutathione extrusion from red cells as GSSG and loss of antioxidant enzymes through the dialyzer membranes, or enhancement of consumption for counteracting OS [17]. This is associated with the increase in MCHC; as a result of loss of erythrocyte water content, which leads to a local increase in RBC cytoplasmic viscosity resulting from increased MCHC and loss of deformability [22].

The present study revealed statistically significant inverse correlation between GSH-Px and GSH-Rx versus BMI and positive correlation with duration of HD therapy; this is in line with Köken et al., study [23].

In contrast Ogunro and his colleagues [24]; found non-significant lower GSH values in the post-HD compared to pre-HD groups while Ozden et al [17]; demonstrated higher GSH-Px levels after HD than before HD.

In this study; PS membrane dialyzers were used for HD, which may explain the reduced activities of the GSH-Px. However, other studies [25-26] observed insignificant differences in the activities of the GSH-Px in any group during the HD procedure while using different dialysis membranes.

In the present study, patients received EPO therapy with parenteral iron have higher red cell GSH-Px enzyme activity compared to patients receiving iron therapy alone, this can be explained by the fact that red blood cells are themselves a circulating antioxidant system due to their content of reduced glutathione and other antioxidant enzymes [27]. Many authors established that EPO have a potential antioxidative effect in HD patients as the correction of anemia in uremic patients, besides its primary beneficial effects represents an effective approach to reduce OS [28-31].

In the present study; multivariate regression analysis showed that red cell GSH-Px decreases with the increase of HD therapy duration which potentiates the effects of OS. This is concordant with other studies [4, 32].

In the present study; multivariate regression analysis showed that HD therapy duration, BMI, serum urea, creatinine, total proteins and Hct are significant factors affecting red cell GSH-Px level, also Pearson correlation demonstrated statistically significant inverse correlation between GSH-Px versus serum creatinine urea and BMI. Whereas serum urea, creatinine, neutrophils count, total proteins and red cell parameter MCV and Hct are significant factors affecting red cell GSH-Rx activity and Pearson correlation displayed statistically significant positive correlation between GSH-Rx versus MCV, which accord with Waggiallah and Alzohairy study as red cell GSH-Rx deficiency has significant correlation with low hemoglobin concentration and low concentration of red cell count and red cell indices: MCV, MCH and MCHC [33].

ROC curve examining the values of red cell GSH-Px and GSH-Rx in HD patients showed that cut off value [measured by the area under the curve (AUC) of 1.0], which discriminate between HD patients and normal control is (86) and (69) (nmol/min/ml) respectively with sensitivity 100% and specificity 100%.

ESRD patients on regular HD had a marked reduction in red cell antioxidant enzymes (GSH-Px and GSH-Rx) activities before HD with further reduction of the enzyme levels after HD i.e. HD dialyzer membranes exert an oxidative stress on red cells that was already exhausted due to continuous exposure to the uremic milieu and frequent dialysis procedure that augment anemia. This is more evident with extended duration of therapy on HD. therefore red cells GSH-Px and GSH-Rx enzymes measurements may serve as a reliable marker for monitoring oxidative stress during HD. It is suggested that erythropoietin together with antioxidant supplementation and the use of membrane coated with antioxidant vitamin E are capable of improving antioxidant defense thus preventing oxidative damage induced by HD.

Strengths of this study are that its preliminary study investigate the effect of HD Session and anemia on Red Cells Antioxidant Enzymes (Glutathione Peroxidase and Reductase) in ESRD Patients. Limitations of this study are that it can't be generalized beyond the study setting, limited number of patient's and further longitudinal studies involving larger number of patients are needed.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgment

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