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Plasma Retinol and Malondialdehyde Levels among Hemodialysis Patients

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ABSTRACT: ***Background: Increased oxidative stress is a well-known phenomenon in dialysis patients. Oxidative stress is viewed as a disturbance in the balance between oxidant production and antioxidant defense. Retinol, the major circulating form of vitamin A, was shown to have some antioxidant properties. Increased oxidative stress may play a role in morbidity and mortality of patients with end stage renal disease. Objective: The aim of the study was to measure oxidative stress markers and antioxidant enzymes in hemodialysis (HD) patients, and compare these findings with those with no renal disease. Methods: The study included 50 subjects conducted from outpatients of Nephrology and Dialysis Unit, Internal Medicine Department, Zagazig University Hospitals. Participants were enrolled after written informed consent and were divided into3 groups: Group I(Control):10 healthy volunteers, Group II: 20 patients with chronic renal impairment on conservative treatment. Group III:20 patients with ESRD for HD and blood samples were taken before the first HD session then after 3months of regular HD. Plasma retinol and malondialdehyde (MDA)levels were quantified; blood activities of catalase (CAT),superoxide dismutase (SOD)and δ-aminolevulinate dehydratase (ALA-D) were analysed. Results: Plasma retinol levels and MDA were high significantly increased in HD group after dialysis compared to the patients before dialysis as regards the mean±SD of plasma retinol and MDA levels; the biomarker of lipid peroxidation. Also, the SOD activity and CAT activity were high significantly increased in patients after dialysis when compared to the patients before dialysis as regards the mean±SD of SOD and CAT. On the other hand, the ALA-D activity was high significantly decreased in the patients after dialysis when compared to patients before dialysis as regards the mean±SD of ALA-D. Also, there was high significant positive correlation of plasma retinol with MDA,SOD and CAT in group III after HD. Conclusion: There was a significant difference in antioxidant capacity before and after hemodialysis.***

Keywords: hemodialysis, MDA, oxidative stress, ALA-D activity, plasma retinol

1. Introduction

Chronic kidney disease (CKD) is an important challenge for health systems around the world consuming a huge proportion of health care finances. It is even more significant for developing countries and morbidities and mortalities emanating from CKD in these countries are immense and related to limited access for treatment options **[1].**

End stage renal disease (ESRD) patients have a well recognized risk of cardiovascular disease that begins early in the course of CKD and results in a tenfold or higher cardiovascular mortality rates after the start of renal replacement therapy [**2**]. ESRD is one of the most common chronic diseases which its incidence and prevalence are on the rise [**3**].

Chronic renal failure (CRF) is a pro-oxidant state and the degree of intracellular and extracellular oxidative stress is related to the severity of renal failure [**4**]. Hemodialysis (HD) represents a state of chronic stress for the patient where the oxidative reactions are mainly due to bioincompatibility of components of dialysis apparatus leading to the production of ROS by inflammatory cells [**5**].

Maintenance hemodialysis (MHD) patients are known to have excessive oxidative stress burden and inflammation **[6].** Oxidative stress has been defined as a loss of balance between the production of free radical or reactive oxygen species (ROS) and protective antioxidant systems**.** A profound imbalance between oxidants and antioxidants has been suggested in uremic patients on maintenance hemodialysis patients [**7**].

In HD patients, increased ROS production is present due to uremia *per se*. Oxidative stress is further aggravated by chronic inflammation, diabetes, advanced age, and losses of small molecules, such as the antioxidant ascorbate, into the peritoneal cavity along a concentration gradient between plasma and peritoneal dialysis (PD) fluids [**8**].Therefore, even in the presence of vitamin E status that is higher compared to healthy control subjects; HD patients still exhibit an oxidant–antioxidant imbalance, as evidenced by elevated plasma concentrations of malondialdehyde (MDA), an end product of the peroxidation of polyunsaturated fatty acids with three or more conjugated double bonds [**9**].

Plasma retinol concentrations have been estimated in patients on maintenance hemodialysis over a 4-year period. For the first 2 years multivitamin supplements containing vitamin A were taken, and for the second 2 years no vitamin A supplements were given. Mean plasma retinol concentrations decreased significantly but only from 3.8 times normal to 3.1 times normal after vitamin A supplements stopped [**10**]. It is important to clarify that δ-ALA-D activity is decreased in CRF, especially during HD treatment. Furthermore, malondialdehyde (MDA) is a more specific and sensitive biomarker for the evaluation of the lipid peroxidation status in many pathologies, including in patients under chronic HD treatment [**11**]. Therefore, the aim of this investigation was to study associations and possible interactions between antioxidant status, biomarkers of

oxidative and glycoxidative stress, and inflammation in HD patients. So, we measured oxidative stress markers and antioxidant enzymes in hemodialysis patients, and compared these findings with those with no renal disease.

2. Subjects and Methods

This study has been conducted on 50 subjects and 20 of them were with CRF undergoing regular HD treatment from outpatient of Nephrology and Dialysis Unit, Zagazig University Hospitals during the period from April 2013 to December 2013. Participants were enrolled after written, informed consent prior to their inclusion in the study. Included subjects of this study were non diabetic, non-smokers, free from hepatic disorders, acute inflammatory conditions or acute infections and not receiving antioxidant supplements or drugs that are known to affect the oxidative state. Subjects under 19 years, using vitamin D, erythropoietin and statins were excluded from the study.

2.1 Subjects

Studied subjects were divided into three groups: **Group I** (Control group): included 10 healthy volunteers (5 males and 5 females) with age ranged from 29 to 55 years with mean values ± SD of 45.9±7.7 years, **Group II**: included 20 patients with chronic renal impairment on conservative treatment (8 males and 12 females) with age ranged from 19 to 68 years with mean values ± SD of 50.9±12.6 years and **Group III**: included 20 patients with diagnosis of end stage renal disease for hemodialysis (7 males and 13 females) with age ranged from 28 to 75 years with mean values ± SD of 48.5±15.3 years, blood samples were taken before the first hemodialysis session (pre-dialysis patients) then after three months of regular hemodialysis (post-dialysis patients) for the same group of patients.

All patients history was carefully recorded by interview and confirmed by checking patient's records and also recording drug prescription (past history of diseases and type of medication received with special attention for nephrotoxic agents). Patient history also include: the weight of the subjects in light outdoor clothes without shoes; special habits of medical important (smoking, alcohols & drug addiction); arterial blood pressure (systolic and diastolic blood pressure) was measured after resting for 5 minutes; symptoms and signs of chronic renal failure; cardiovascular risk factors and complications, and pulmonary risk factors and complications.

2.2Routine investigations for all subjects include

1. Complete blood count (CBC).
2. Urine analysis.
3. Fasting and post prandial blood glucose
4. Lipid profile including: Serum triglycerides, total cholesterol level, High density lipoproteins (HDL) and Low density lipoproteins (LDL)**.**
5. Liver and kidney function tests
6. Serum uric acid**.**
7. Creatinine clearance is used as correlation of GFR and estimated by using **Cockcroft and Gault** equation**[12]**:

$$\text{Creatinine clearance} = \frac{(140 - \text{age (yr)}) \times \text{body weight } (kg)}{\text{Serum creatinine (mg/dl)} \times 72},$$

Female: × 0.85

8. Pelvi-abdominal ultrasonography: included comments on both kidneys size, degree of renal parenchymal echogencity, parenchymal thickness, differentiation between cortex and medulla and presence of renal cysts or stones.
9. Chest X-ray: plain chest X-ray films were examined for any chambers enlargement, pulmonary edema and pleural effusion.
10. Resting 12 leads electrocardiographic tracing.

2.3 Special investigations included:

- Plasma retinol.
- Lipid peroxidation biomarker: malondialdehyde (MDA).
- Antioxidant enzymes: superoxide dismutase (SOD) and catalase (CAT) levels.
- Erythrocyte ALA-D activity: δ- aminolevulinate dehydratase.

Samples

Venous blood samples (8 mL) were drawn from subjects, the collection was held during the morning. Then, these samples were divided into heparinized tubes, EDTA-containing tubes and tubes without anticoagulant. Plasma and serum were obtained by centrifugation at 1500 g for 10 min at 4◦C. In hemodialysis group, samples were drawn before the first hemodialysis session then after three months of regular hemodialysis.

Retinol Assay:

Plasma retinol level determination using HPLC**:** Measurements were made using HPLC100(Hewlett-Bacard,Waldbronn-Germany) equipped by UV detector , data analysis and elaboration were processed by LC-Chem .station soft ware. standard provided by Sigma-Aldrich Chemie GmbH

Specimens preparation

100 µl of plasma was taken, and transferred into a conical glass centrifuge tube of 5 ml capacity. 100 µl retinyl acetate (internal standard) in absolute ethanol was added to this tube. The contents were mixed on a vortex mixer for exactly 15 seconds, and then the tube was transferred to an icebox for minimum 5 minutes. Finally, 1 ml of n-hexane was added to the tube, and the contents were mixed on a vortex mixer for 1 min exactly. The tube was centrifuged at 25°C for 5 minutes and the upper clear hexane layer was carefully drawn in another test tube (5 ml capacity). Hexane was then evaporated under a gentle stream of nitrogen gas. After the evaporation of hexane was complete, 100 µl of methanol was added, and the contents were mixed on a vortex mixer for a minimum of 15 seconds. The aliquot, thus prepared, was injected into the HPLC loop using a Hamilton microliter syringe. The injection volume was 20 µl.

Quantitative Measurement

Internal standard was added to the sample at the beginning of the extraction procedure to compensate for losses at each step of the sample preparation. After processing, the final extract was filtered with a 4 mm, 0.45µm disposable membrane filter and then chromatographed for the simultaneous determination for retinol. Calibration curves were made by determining peak height ratios relative to the internal standard. With internal standard data analysis, the system controller software automatically adjusts the amount of the analyte in unknown samples, in relation to the amount of internal standard added at the beginning of the whole procedure.

Estimation of Lipid Peroxidation Products: by Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit ,Plasma MDA in the sample is reacted with Thiobarbituric Acid (TBA) to generate the MDA-TBA adduct which can be easily quantified colorimetrically (OD 532 nm).

Estimation of Superoxide dismutase (SOD): by Abcam's Superoxide dismutase activity assay kit ,using WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, the inhibition activity of SOD can be determined spectrophotometrically at 450 nm.

Preparation of haemolysates for SOD assay : After separation of plasma, the packed erythrocytes were washed 3 times with an isotonic saline Packed blood cells were lysed by addition of equal volume of cold deionised water; Centrifuge at 12000 x g for 10 minutes and collect the cell lysate supernatant prior to SOD assay.

Estimation of Catalase (CAT):

Catalase measurement will be determined based on the ability of catalase to oxidize hydrogen peroxide. Briefly the method is as follows: 2.25 ml of potassium phosphate buffer (65 mM, pH 7.8) was added to 0.1 ml of serum and incubated at 25°C for 30 min. 65 ml of hydrogen peroxide (7.5 mM) were added to initiate the reaction. The change in absorbance was measured at 240 nm for 3 min. One international unit (IU) of catalase is the enzyme, which decomposes one mM of hydrogen peroxide per min at 25°C14.

Estimation δ-Aminolevulinate dehydratase (δ-ALA-D): provided by Sigma-Aldrich Chemie GmbH**,** δ-Aminolevulinate dehydratase activity will be determined in the total blood, with heparin as anticoagulant, . The enzyme activity was determined by the rate of porphobilinogen (PBG) formation in 1 h at 37° C, in the presence and absence of the redactor agent dithiothreitol (DTT 2 mM final concentration). The enzyme reaction was initiated after 10 min of pre-incubation. The reaction was started by adding δ-aminulevulinic acid (ALA) to a final concentration of 4 mM in a phosphate buffered solution at pH 6.8; incubation was carried out for 1 h at 37◦C and the reaction product was measured at 555 nm.

2.4 Statistical Analysis

Statistical computations were performed with the Statistica® 6.0 software system (**Statsoft Inc., 2001**). The results are expressed as mean ± standard error medium (SEM). Comparisons between groups were achieved by the Student *t*-test or Mann–Whitney test, depending on the variable distribution. Pearson's correlation or Spearman's rank order correlation was used to evaluate the relationship between pairs of variables, following the variable distribution. Partial correlation was used to evaluate the relationship between a pair of variables while controlling for a third variable. A value of $P<0.05$ was considered significant.

3. Results

The demographic characteristics and clinical data in the different groups of the study are presented in **Table 1**. The result showed that, there was non-significant difference among the different groups of the study as regard to the mean values ± SD of the age (years) ($F = 0.58$, $p > 0.05$) and gender ($\chi2 = 0.63$, $p > 0.05$). Also, there was a statistically significant difference as regards systolic blood pressure (mm Hg) ($F=6.28$, $p < 0.05$) and diastolic blood pressure (mm Hg) ($F=4.49$, $p < 0.05$).

Table 2 presents the comparison of kidney function tests among the studied groups. Our finding showed that, there was statistically high significant difference in the mean values ± SD of blood urea (mg/dl) ($F=88.8$, $P<0.001$), serum creatinine (mg/dl) ($F=58.40$, $p<0.001$) and creatinine clearance (ml/min/1.73 m^2) ($Kw = 42.5$, $p<0.001$). Also, there was significant difference in the mean values ± SD of serum uric acid (mg/dl) ($F=5.87$, $P<0.05$) among the studied groups compared to control group.

Table 3 summarizes the mean values ±SD of some specific biochemical parameters such as (plasma retinol concentration, MDA, SOD, CAT and ALA-D) in studied groups compared by control group. Our finding showed that, a statistically high significant difference was found as regards the mean values ± SD of plasma retinol (µmol/L) ($F=23.3$, $p<0.001$), MDA (nmol\L) ($F=41.7$, $p<0.001$), SOD (U/L) ($F=21.4$, $p<0.001$) CAT (mmol/L) ($F=18.1$, $p<0.001$) and ALA-D(IU/L) ($F=99.2$, $p<0.001$) among studied groups compared to control group.

Table 4 presents the liver function tests include (ALT, AST, and ALB) and kidney function tests include (blood urea, serum creatinine, and serum uric acid) in group III (pre- and post-dialysis). There was non-statistical significant difference as regard to the mean values ± SD of ALT (U/L) ($t=0.07$, $p>0.05$), AST (U/L) ($t=0.7$, $p>0.05$) and ALB (gm/dl) ($t=0.0$, $p>0.05$) in hemodialysis group after dialysis as compared to before dialysis in the same group. As regards, there was statistically high significant decrease in the mean values ± SD of blood urea ($t= 10.2$, $p<0.001$), serum creatinine ($t= 7.4$, $p<0.001$) and serum uric acid ($t= 9.9$, $p<0.001$) in hemodialysis patients of group III after dialysis as compared to before dialysis in the same group.

Table 5 presents comparisons of specific biochemical parameters including plasma retinol, MDA, SOD, CAT, and

ALA-D. This table shows that the plasma retinol levels and plasma malondialdehyde (MDA) were high significantly increased in hemodialysis group after dialysis when compared to the patients before dialysis as regard to the mean values ± SD of plasma retinol (µmol/L) (t=6.49, p<0.001) and plasma malondialdehyde (MDA) levels, the biomarker of lipid peroxidation (nmol\L) (t=11.58, p<0.001). Also, this table shows that the superoxide dismutase (SOD) activity and catalase (CAT) activity were high significantly increased in the patients after dialysis when compared to the patients before dialysis as regard to the mean values ± SD of SOD (U/L) (t=6.21, p<0.001) and CAT (mMol/L) (t=4.96, p<0.001). on the other hand, The δ-aminolevulinate dehydretase (ALAD) activity was high significantly decreased in the patients after dialysis when compared to the patients before dialysis as regard to the mean values ± SD of ALA-D(IU/L) (t=11.58, p<0.001).

Table 6 presents the correlation coefficients of plasma retinol concentration versus MDA, SOD, CAT, and ALA-D levels in group III after hemodialysis. There was a statistically higher significant positive correlation of plasma retinol with MDA (nmol\L) (r =0.66, p<0.001), SOD (U/L) (r = 0.48, p<0.001) and CAT (mmol/L (r = 0.52, p<0.001). while there was high significant negative correlation between it and ALA-D (IU/L) (r = -0.65, p<0.001) in group III after hemodialysis.

Table 1: Demographic and clinical data among the studied groups

Variables	*Group I (control) (n = 10)*		*Group II (chronic renal impairment) (n = 20)*		*Group III (pre-dialysis) (n = 20)*		*P value*
Age (years) X±SD Range	45.9±7.7 (29-55)		50.9±12.6 (19-68)		48.5±15.3 (28-75)		0.56
Gender	**No**	**%**	**No**	**%**	**No**	**%**	
Male	5	50.0	8	40.0	7	35.0	0.73
Female	5	50.0	12	60.0	13	65.0	
SBP (mm Hg) X±SD Range	114.0±10.7 (100-130)		138.0±24.0 (110-160)		142.5±22.4 (100-180)		0.003
DBP (mmHg) X±SD Range	77.0±4.5 (70-80)		86.5±10.3 (70-105)		86.7±9.3 (70-100)		0.016

SBP: Systolic blood pressure **DBP**: Diastolic blood pressure **%**: Percentage Significant (p value < 0.05). Highly significant (p value < 0.001) Non significant (p value > 0.05)

Table 2: Comparison of different parameters among studied groups.

Parameters	*Control (n = 10)*	*Group II (n = 20)*	*Group III (n = 20)*	*P value*
Blood Urea (mg/dl) X±SD Range	23.2±3 (18-28)	94.8±25.3 (66-144)	254.3±73.8 (200-537)	<0.001
SCr (mg/dl) X±SD Range	0.8±0.1 (0.67-1.1)	3.35±0.9 (2-5.1)	11.9±4.7 (7.1-24)	<0.001
CCl (ml/min/1.73 m^2) X±SD Range	138.0±21.5 (103-170)	22.2±8.3 (11-38)	5.4±1.6 (2-7)	<0.001
Serum Uric acid (mg/dl) X±SD Range	5.0±1.5 (3.4-7.2)	5.8±1.1 (4.1-8.2)	6.6±1.2 (5-10.5)	0.005

S: Significant (p value < 0.05). **SCr:** serum creatinine **CCl:** creatinine clearance

Table 3: Comparison of specific biochemical parameters (plasma retinol, MDA, SOD, CAT and ALA-D) among studied groups.

Parameters	*Control (n = 10)*	*Group II (n = 20)*	*Group III (n = 20)*	*P value*
Retinol (µmol/L) X±SD Range	2.1±0.12 (1.94-2.36)	2.85±0.5 (2.3-3.59)	3.78±0.9 (2.5-5.58)	<0.001
MDA (nmol\L) X±SD Range	38.8±20.8 (16.95-78.97)	123.2±38.4 (74.2-218.6)	161.4±30.9 (85.71-224.39)	<0.001
SOD (U/L) X±SD Range	33.8±6.78 (26.5-48)	38.3±5.4 (25.8-50.4)	46.8±5.1 (37.8-58.2)	<0.001
CAT (mmol/L) X±SD Range	33.1±10.6 (33.46-52.17)	94.3±42 (40.37-184.66)	128.2±47.56 (47.41-198.86)	<0.001
ALA-D (U/L) X±SD Range	19.6±0.89 (18.32-20.7)	16.9±0.96 (14.63-18.74)	14.99±0.68 (14.32-16.63)	<0.001

MDA: Malondialdehyde **SOD**: Superoxide dismutase **CAT:** Catalase **ALA-D**: Aminolevulinate degydratase

Table 4: Comparison of liver function tests (ALT, AST and ALB) and kidney function tests (blood urea, SCr, CCl, and serum uric acid) in hemodialysis group III pre-and post-dialysis.

Parameters	*Pre-dialysis*	*Post-dialysis*	*P*
ALT (U/L) X±SD Range	20.5±7.0 (12-40)	20.5±7.2 (7-35)	0.94
AST (U/L) X±SD Range	21.7±6.4 (3-33)	21.6±5.2 (13-29)	0.94
ALB (gm/dl) X±SD Range	3.5±0.3 (2.8-4.2)	3.5±0.19 (3.1-4)	1.0
Blood Urea (mg/dl) X±SD Range	254.3±73.8 (200-537)	98.1±12.3 (79.-125)	<0.001
SCr (mg/dl) X±SD Range	11.9±4.7 (7.1-24)	4.87±1.0 (3.2-7.5)	<0.001
Serum Uric acid (mg/dl) X±SD Range	6.6±1.2 (5 - 10.5)	4.9±0.65 (4.2 - 6.5)	<0.001

ALT: Alanine aminotransferase **AST**: Aspartate aminotransferase. **ALB**: Albumin **SCr**: Serum creatinine

Table 5: Comparison of specific biochemical parameters in hemodialysis group pre-and post-dialysis

Parameters	*Pre-dialysis*	*Post-dialysis*	*P*
Retinol (µmol/L) X±SD Range	3.78±0.9 (2.5-5.58)	5.5±1.0 (3.47-8.87)	<0.001
MDA (nmol/ml) X±SD Range	161.4±30.9 (85.71-224.39)	206.8±56.6 (134.84-303.91)	<0.001
SOD (U/L) X±SD Range	46.8±5.1 (37.8-58.2)	55.7±5.9 (47.4 - 64.8)	<0.001
CAT (mmol/L) X±SD Range	128.2±47.5 (47.41-198.86)	164.1±33.5 (102.27-225.9)	<0.001
ALAD (U/L) X±SD Range	14.99±0.68 (14.32-16.63)	12.1±0.8 (11.0-13.41)	<0.001

MDA: Malondialdehyde **SOD**: Superoxide dismutase **CAT:** Catalase **ALA-D**: Aminolevulinate dehydretase

Table 6: Correlation coefficient of plasma retinol (µmol/L) versus some studied parameters in group III after hemodialysis

Item	*Retinol (µmol/L)*	
	r	*P*
MDA **(nmol/ml)**	0.66	< 0.001
SOD **(U/L)**	0.48	< 0.001
CAT **(mmol/L)**	0.52	< 0.001
ALA-D (U/L)	-0.65	< 0.001

MDA: Malondialdehyde **SOD**: Superoxide dismutase **CAT:** Catalase **ALA-D**: Aminolevulinate dehydretase

4. Discussion

Chronic renal failure is a pro-oxidant state, characterized by increased levels of free radical oxidants relative to antioxidants [**13**]. Hemodialysis leads to significant changes in the antioxidant system of the blood of patients with CRF. The effect is noticeable with antioxidant enzyme activities and concentrations of nonenzymatic components of the system [**14**]**.** Retinoids have redox-related properties and they influence the oxidative status of the cell. Increased concentrations of malondialdehyde (MDA)-intermediate product of oxidation of polyunsaturated fatty acids have been reported in the plasma and erythrocytes, as well as in platelets and mononuclear cells of hemodialyzed patients [**15**].

The aim of the present work was designed to study the possible influence of plasma retinol levels on classical oxidative stress blood biomarkers as antioxidant enzymes activity include: (SOD and CAT levels), lipid peroxidation biomarkers include: malondialdehyde (MDA) and erythrocyte ALA-D activity include: δ-aminolevulinate dehydratase in HD patients before and after dialysis compared to healthy subjects and chronic renal impairment patients. Also, we evaluate the effect of dialysis on oxidative stress.

In both chronic renal impairment and hemodialysis patients the systolic and diastolic blood pressure were significantly higher than the control group that may point to a pathogenic role of hypertension, among other factors, in the development of vascular and renal diseases. In both chronic renal impairment and hemodialysis patients serum uric acid, serum creatinine and blood urea were significantly higher than control group. Also, serum creatinine and blood urea were significantly increased in hemodialysis group before the first session compared with chronic renal impairment patients. Both uremic markers were significantly decreased in patients after hemodialysis compared with the patients before dialysis. Creatinine clearance was significantly decreased in studied groups compared with control group.

Low clearance values for creatinine and urea indicate a diminished ability of the kidneys to filter these waste products from the blood and to excrete them in the urine. As clearance levels decrease, blood levels of creatinine and urea nitrogen increase. Since it can be affected by other factors, an elevated blood urea alone is certainly suggestive for kidney dysfunction. However, it is not diagnostic. An abnormally elevated blood creatinine, a more specific and sensitive indicator of kidney disease than the BUN, is diagnostic of impaired kidney function [**16**].

The levels of plasma retinol were highly increased in hemodialysis patients after three months of regular dialysis when compared to the same patients before hemodialysis. Plasma malondialdehyde (MDA) levels, the biomarker of lipid peroxidation, were also high significantly increased in patients after hemodialysis compared with the same patients before hemodialysis. This result is in agreement with **Roehrs et al** who reported in their results, plasma retinol levels have significantly increased in HD patients compared to healthy subjects [**17**]. Also, they reported that the plasma MDA levels, the biomarker of lipid peroxidation, were significantly increased in HD patients. There was a positive correlation between plasma levels of retinol and MDA, this supports this connection between CKD and oxidative stress. There is increasing evidence that oxygen radicals are involved in the progression of renal damage and of uremic symptoms [**18**].

Lipid peroxidation products (MDA and 4-Hydroxnonenal) were higher in CRF patients under hemodialysis compared with those on conservative treatment though this difference did not reach a significant value. This difference in lipid peroxidation markers in hemodialysis patients than in CRF patients on conservative treatment suggests that hemodialysis may be a cause of oxidative stress due to the activation of polymorphonuclear neutrophil leukocytes (PMNLs) through contact of blood with the dialysis membranes **[19]**.

Hemodialysis may induce repetitive bouts of oxidative stress, primarily through membrane bioincompatability and endotoxin challenge. While alterations in pro- and anti-oxidant capacity start during the early stages of CRF, they are most pronounced in patients who are on dialysis. Overall, there is some controversy as to whether the onset of regular dialysis improves or worsens oxidative stress [**20**]**.**

Taccone-Gallucci, et al. observed evidence for elevated lipid peroxidation in the erythrocyte membranes of patients with serum creatinine > 5.0 mg/dl. It is interesting that these patients were not on dialysis. This suggests that the lipid peroxidation abnormality is related to renal failure rather than the process of dialysis [**21**].

In this study, activities of erythrocyte enzymes that scavenge superoxide radicals (SOD) and catalase (CAT) were measured in HD patients and controls. These results demonstrated highly significant increase of the blood SOD and CAT activity in patients after dialysis when compared to the patients before hemodialysis. Also, superoxide dismutase (SOD) activity and catalase activity (CAT) were significantly increased in studied groups when compared to controls. **Roehrs and his workers** suggested that there was significantly increased in SOD activity in HD patients compared to healthy subjects [**17**]. There was a positive correlation between plasma levels of retinol and superoxide dismutase (SOD). Also, there was a positive correlation between plasma levels of retinol and catalase (CAT)

The aminolevulinate dehydratase (ALA-D) activity also was significantly decreased in patients before hemodialysis compared with patients after dialysis. The aminolevulinate dehydratase (ALA-D) activity also was significantly decreased in dialysis group compared to healthy group. There was a negative correlation between plasma levels of retinol and ALA-D in the patients after hemodialysis.

The present study showed that plasma retinol levels and plasma malondialdehyde (MDA) levels were significantly increased in dialysis patients compared to healthy subjects. Also, our study showed that the SOD-activity was significantly higher in dialysis patients compared to healthy subjects, and CAT activity also was significantly higher in dialysis patients compared to healthy subjects. These results are in accordance with those of older studies [**22,23**].

Several publications describing enzyme participation in free radical metabolism have yielded wrong and mixed results. **Roehrs et al** explained this by adaptative mechanisms to oxidative stress [**17**]. This mechanism can also be explained by another finding, in accordance with **Murata and Kawanishi,** who demonstrated the increase of O_2^- and H_2O_2 by retinol increase [**24**]. Our results tend to confirm these observations. The activities of these enzymes (CAT and SOD) were significantly with the retinol levels.

Failure of the kidney to metabolize retinol to retinoic acid may cause diminished excretion via bile and urine, thus leading to accumulation of retinol in chronic renal failure (CRF) [**25**].

In this study our result also, found that an increased with retinol, MDA, SOD and CAT parameters compared with ALA-D had decreased after dialysis. In contrast, the HD procedure had no significant effect on plasma retinol level. As it is a lipid soluble compound, so significant dialytic clearance of plasma retinol is not expected [**26**].

Ajala et al. in his study, found that a significant increase in MDA were observed, which is the measurement of secondary products of lipid peroxidation and a significant decrease in total antioxidant status in chronic renal failure patient after dialysis [**27**].

This finding supports most of the previously published findings that the hemodialysis procedure alters lipid peroxidation and the antioxidant status. Some researchers have reported increased status [**28**], while some have recorded reduction [**29**], and others were equivocal [**30**] on MDA levels after hemodialysis. However, **Peuchant et al.** (**1994**) reported low post-hemodialysis MDA in contrast to increased MDA reported in studies where less specific analytical techniques were used [**31**].

Our study demonstrated that before dialysis patients had low MDA levels and high antioxidant status, but after dialysis samples gave an opposite levels of the analytes. In study done by **Derici U et al.** of patients with chronic renal failure that had peritoneal dialysis, they observed high MDA levels and low antioxidant capacity [**32**]. There seems to be no significant difference in the serum level of oxidative stress indices between peritoneal dialysis and hemodialysis patients regardless of the structure of the dialysis membranes used. The use of vitamin E-coated membranes could help minimize the increased oxidative stress and the attendant risk of atherosclerosis in dialysis patients. **Mune et al.** have shown that the use of vitamin E-coated cellulose membrane dialyzers for 6 months resulted in a significant reduction in low density lipoprotein, oxidized-LDL, and eventually low peroxidation compared to the ordinary cellulose membrane dialyzer [**33**]. In order to decrease membrane bioincompatibility, and thereby minimize oxidative stress in hemodialysis patients, more compatible filters have been elaborated. Preliminary characterization of vitamin E-coated membranes has shown decreased activation of polymorphonuclear cells and monocytes, lower free radical production, and high biocompatibility [**27**]. Due to this conflict of information, more studies regarding the action of retinol are still needed since there are several therapies based on the use of retinol for several diseases [**34**].

5. Conclusion

From the present study we could conclude that there was a significant difference in antioxidant capacity before and after hemodialysis. There is loss of antioxidant during the course of hemodialysis, probably through the dialyzer membranes or generation of free radicals on the surface of dialyzer membranes or both. The decreased antioxidant capacity could be related to increasing lipid peroxidation in hemodialysed patients as there was a positive correlation between high plasma retinol levels and lipid peroxidation and also the induction of antioxidant enzyme activity and inhibition of thiol group-dependent enzyme, ALA-D, increase of plasma retinol levels in HD patients tends to act as additional effect, as a pro-oxidant agent.

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