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Original Article

Oxidative Stress in Uremic Children and its Relation to Hemoglobin Levels and Left Ventricular Hypertrophy

Al-Banna H., Atfy M., Osman M., Hasan S.* and Kamel E.

Departments of Pediatrics and Medical Biochemistry, Faculty of Medicine, Zagazig University, Egypt*

ABSTRACT

Background: Cardiovascular diseases (CVD) represent the major cause of mortality in hemodialysis patients. Several factors are supposed to be responsible for that increase in cardiovascular risks. Oxidative stress has been postulated to be an important risk factor for CVD. Oxygen free radicals and their secondary products, together called reactive oxygen species (ROS), exert angiotoxic and cardiotoxic effects.

Objectives: To evaluate serum levels of lipid peroxidation (LPO) products, 4-hydroxynonenal (HNE) and malondialdhyde (MDA), as indicators of oxidative stress in children with chronic renal failure (CRF) before and after dialysis and their relation to hemoglobin (Hb) level and left ventricular hypertrophy (LVH).

Methods: This cross-sectional study included 20 children (8 males and 12 females) suffering from CRF on conservative therapy only without taking erythropoietin (non dialyzed group) and 20 children (8 males and 12 females) suffering from CRF on regular hemodialysis and erythropoietin (EPO) therapy for at least 6 months (dialyzed group). Their ages ranged from 4 to 16 years. 20 healthy children of comparable age and sex were taken as a control group. Measurement of MDA and HNE as markers of lipid peroxidation (LPO) was performed by using spectrophotometry and high performance liquid chromatography, respectively. Echocardiography was done for all subjects as well.

Results: There was an increase in LPO products (HNE & MDA) in CRF patients compared with control subjects. However, HNE only was significantly higher in group I (non-dialyzed) than group II (dialyzed); 353.27 ± 55.5 vs. 346.7 ± 58.8, $p < 0.01$; while MDA levels in both group I and group II showed non-significant difference; 4.4 ± 1.6 vs. 4.3 ± 1.3, $p > 0.05$. Hemoglobin levels were significantly lower in CRF patients than control subjects, but Hb was non-significantly higher in group II (dialyzed) than group I (non-dialyzed); 8.7 g/dl ± 2.5 vs. 8.2 ± 2.3, $p < 0.05$. Left ventricular mass index (LVMI) was significantly higher in CRF patients than control subjects. However, LVMI was significantly higher in group II (dialyzed) than group I (non-dialyzed); 118.1± 36.88 vs. 110.13 ± 5.15, $p < 0.05$. LVMI, HNE and MDA were all higher in CRF patients with Hb levels less than 9.5 gm/dl than those with Hb levels higher than 9.5 gm/dl. A significant negative correlation was found between Hb levels and LPO (HNE & MDA) as well as LVMI; $r = -0.75, -0.81, -0.65$; respectively. A significant positive correlation was found between LPO products (HNE & MDA) and LVMI; $r = 0.54, 0.41$, respectively.

Conclusions: Oxidative stress and lipid peroxidation represent a major health problem in CRF patients. Optimized correction of anemia as well as regular efficient dialysis help reduce oxidative stress including lipid peroxidation, thereby reducing cardiovascular morbidity in patients with CRF, thus improving their quality of life and improving the prognosis of renal failure.

INTRODUCTION

Cardiovascular diseases (CVD) represent the major cause of mortality in hemodialysis patients. Several factors are supposed to be responsible for that increase in cardiovascular risks[1]. Oxidative stress has been postulated to be an important risk factor for CVD. Oxygen free radicals and their secondary products, together called reactive oxygen species

(ROS), exert angiotoxic and cardiotoxic effects. Consequently; these compounds contribute to increased cardiovascular risks[2]. It is generally accepted that renal failure is associated with drastic oxidative stress. Free radicals and oxidative stress i.e. an imbalance between antioxidants and prooxidants contribute to the pathogenesis and progression of acute and chronic renal failure[3]. Free radicals can damage proteins, lipids, carbohydrates and nucleic acids. Oxygen-derived free radicals can easily produce injuries to cell membranes by initiation of polyunsaturated fatty acid peroxidation, inactivation of membrane enzymes and receptors, and protein cross-linking and fragmentation. Several strands of evidence suggest that oxidative processes may be increased in patients with renal failure[4].

Under oxidative stress, lipids and proteins are modified by reactive free radicals. Free radicals-mediated lipid peroxidation is always accompanied by generation of complex pattern of aldehydes e.g. MDA and HNE.

HNE is produced by oxidation of w-6 fatty acids[5]. In plasma, HNE is transported by lipoproteins. It can be oxidised by NADP dependent aldehyde dehydrogenase to carboxylic acid, or reduced by a NADP dependent alcohol dehydrogenase to an alcohol (1, 4 dihydroxy –2 nonene) and then conjugated by glutathione either spontaneously or by glutathione transferase to glutathione conjugate[6].

Accumulation of dialyzable oxidants have been demonstrated in uremic plasma[7]. In hemodialysis, the absence of complete correction of the uremic toxicity together with the unwanted effects of the dialysis therapy, malnutrition and metabolic abnormalities associated with uremia probably account for the increased oxidative stress. Additionally; dialysis depletes antioxidant defense[4].

Anemia not only promotes CVD in end stage renal disease (ESRD), but also promotes oxidative stress. Low hemoglobin and hematocrit values are associated with increased CVD morbidity and mortality in patients with end-stage renal disease[2].

AIM OF THE WORK

The aim of this study was to evaluate serum levels of cardiotoxic lipid peroxidation products (4-hydroxynonenal {HNE} and malondialdehyde {MDA}) as indicators of oxidative stress in uremic children and their relation to hemoglobin levels and left ventricular hypertrophy.

SUBJECTS AND METHODS

This study was performed on 40 children (16 males and 24 females) suffering from chronic renal failure.

They were randomly selected from those uremic children attending the Pediatric Nephrology Unit of Zagazig University Hospitals, from October 2003 to December 2004. Their ages ranged from 4 to 16 years. These 40 children were divided into two groups:

Group I (Non-dialyzed): 20 children (8 males & 12 females) with chronic renal failure on conservative therapy without taking erythropoietin (EPO).

Group II (Dialyzed): 20 children (8 males & 12 females) with end-stage renal failure

on regular haemodialysis and taking supportive therapy and recombinant human erythropoietin for at least 6 months.

Control group: of 20 healthy children with comparable age and sex was included.

Informed consent was obtained from their parents before they were included. Patients with chronic infection, malignancy, active liver disease, liver insufficiency, systemic disease e.g. DM, chronic chest disease, congenital, rheumatic or primary myocardial diseases as well as pericardial effusion were excluded from the study. All cases were subjected to the following:

1. **Full history taking.**
2. **Thorough clinical examination** including: (a) General examination: with special emphasis on vital signs; blood pressure, heart rate, body weight, height, edema of lower limbs and neck vein congestion. (b) Local examination of chest and heart: to exclude children with pericardial effusion, congenital or acquired valvular heart disease, chronic chest disease e.g. chronic obstructive pulmonary disease, pulmonary fibrosis, from the study.
3. **Laboratory investigations:**
   a. Complete blood count and ESR.
   b. Total protein and serum albumin.
   c. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) spectrophotometrically by commercial kits.
   d. Measurement of serum creatinine, blood urea and serum uric acid by commercial kits.
   e. Estimation of lipid peroxidation products 4-hydroxynonenal (HNE) and malondialdhyde (MDA) by chemical methods; using high performance liquid chromatography (HPLC LC –1610), with UV detector operator at 221 nm, acetonitrile as eluent with flow rate 1 ml/min and injection volume 20 ul. The retention time is 4.7 min. for measuring hydroxynonenal (HNE)[8] and spectrophotometry for measuring malondialdhyde (MDA)[9].
4. **Echocardiography:** M-mode, 2-dimensional and Doppler echocardiography were done for all cases and control subjects.

Statistical analysis

Statistical analysis was performed by comparing the studied groups using student t-test, Chi-square, ANOVA and correlation coefficient. Statistical significance was considered $p < 0.05$. Data were expressed as mean ± standard deviation for quantitative variables, number and percentage for qualitative variables. Multiple comparison analysis test (LSD test) was used to detect statistical significance between two means when ANOVA test was significant. All analysis was done by SPSS software version 10.0.

RESULTS

Table 1 shows that there is a non-significant difference between the study groups as regards age, sex, serum ALT and serum AST ($p > 0.05$), while there is a highly significant difference as regards blood pressure, blood urea, serum creatinine and serum albumin in the studied groups; ($p < 0.01$).

Table 2 shows a non-significant difference between patient groups as regards the frequency of hypertension and left ventricular hypertrophy; ($p > 0.05$).

Table 3 shows a significant difference

among the studied groups as regards hemoglobin concentration; ($p < 0.01$). LSD test shows a significant difference between group I and each of group II and the control group, while both group I and group II show significantly lower hemoglobin concentrations than the control group; ($p < 0.01$). Regarding lipid peroxidation products; HNE & MDA; there is a significant difference among the studied groups; ($p < 0.01$). LSD test shows a significant difference between the studied groups. While both group I and Group II showed significantly higher HNE & MDA concentrations than the control group; ($p < 0.01$), HNE concentrations in group I was significantly higher than group II; $p < 0.01$. However, MDA showed a non-significant difference between group I and group II; ($p > 0.05$). LVMI showed a significant difference among the studied groups; ($p < 0.01$). LVMI was significantly higher in group II than group I; ($p < 0.01$). Both group I and group II showed a highly significant increase of LVMI than the control group; ($p < 0.001$).

Table 4 shows that HNE, MDA and LVMI were all significantly higher in patients with hemoglobin concentrations less than 9.5 gm/dl than in patients with hemoglobin concentrations more than 9.5 gm/dl in both Group I and Group II.

Table 5 shows a significant negative correlation between Hb level and each of HNE, MDA & LVMI; ($r = -0.75$, $r = -0.81$, $r = -0.65$, respectively). It also shows a significant positive correlation between HNE and LVMI as well as MDA and LVMI; ($r = 0.54$, $r = 0.41$, respectively).

Table 1: Demographic, clinical and laboratory data in studied groups.

Variable	Patients (n = 40)		Control (n = 20)	p*
	Group I (n = 20)	Group II (n = 20)		
Age (years)	9.25 ± 3.61	10.45 ± 3.66	10.3 ± 3.21	> 0.05
Sex (M/F)	8/12	8/12	10/10	> 0.05**
Bl. Pressure (systolic)	125.75 ± 6.7	130.5 ± 8.4	104 ± 9.9	< 0.01
Bl. Pressure (diaystol)	84.0 ± 6.4	88.0 ± 4.9	66.25 ± 5.0	< 0.01
Creatinine (mg/dl)	4.62 ± 0.36	6.77 ± 0.82	0.62 ± 0.26	< 0.01
Urea (mg/dl)	78.15 ± 7.32	82.0 ± 5.74	28.0 ± 6.67	< 0.01
Albumin (gm/dl)	2.65 ± 0.17	2.63 ± 0.29	3.92 ± 0.39	< 0.01
ALT (IU/L)	28.5 ± 5.1	26.3 ± 3.1	26.7 ± 5.3	> 0.05
AST (IU/L)	24.3 ± 5.3	26.9 ± 4.8	25.8 ± 8.4	> 0.05

* p by ANOVA test

** p by Chi square test

Table 2: Frequency of hypertension and LVH in patient groups.

		Group I (n = 20)	Group II (n = 20)	X^2	p
Hypertensive patients	No	13	15	0.446	> 0.05
	%	65%	75%		
Left ventricular hypertrophy	No	13	16	0.625	> 0.05
	%	65%	80%		

Table 3: The mean hemoglobin, hydroxynonenal, malondialdhyde and left ventricular mass index in the studied groups

	Group I	Group II	Control	p
Hemoglobin (g/dl)	8.2 ± 2.3	8.7 ± 2.5	14.5 ± 2.1	< 0.01
Group I vs. Group II = non-significant;	p > 0.05			
Group I vs. Control = significant;	p < 0.01			
Group II vs. Control = significant;	p < 0.01			
Hydroxynonenal (umol/l)	353.27 ± 55.5	346.7 ± 58.8	26.3 ± 12.5	< 0.01
Group I vs. Group II = significant;	p < 0.05			
Group I vs. Control = significant;	p < 0.01			
Group II vs. Control = significant;	p < 0.01			
Malondialdhyde (umol/l)	4.4 ± 1.6	4.3 ± 1.3	1.02 ± 0.6	< 0.01
Group I vs. Group II = non-significant;	p > 0.05			
Group I vs. Control = significant;	p < 0.01			
Group II vs. Control = significant;	p < 0.01			
LV mass index	110.13 ± 5.15	118.1 ± 36.88	73.6 ± 11.00	< 0.01
Group I vs. Group II = significant;	p < 0.05			
Group I vs. Control = significant;	p < 0.01			
Group II vs. Control = significant;	p < 0.01			

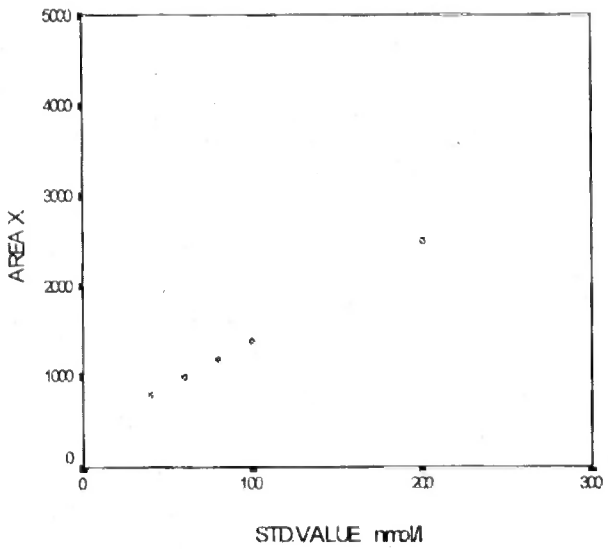
Table 4: HNE and MDA serum levels and left ventricular mass index (LVMI) in relation to hemoglobin concentration in patient groups.

		Hb < 9.5 gm/dl	Hb > 9.5 gm/dl	t	p
Group I	HNE	353.3 ± 21.4	340.5 ± 22.5	2.60	< 0.05
	MDA	5.3 ± 1.7	4.2 ± 1.7	4.48	< 0.01
	LVMI	117.8 ± 41.5	97.03 ± 28.9	4.59	< 0.01
Group II	HNE	331.1 ± 34.5	304.6 ± 31.4	12.93	< 0.01
	MDA	4.4 ± 1.5	3.3 ± 1.3	2.70	< 0.05
	LVMI	131.08 ± 43.9	108.49 ± 17.34	4.65	< 0.01

Table 5: Correlation of HNE & MDA levels with Hb and left ventricular mass index (LVMI) in CRF patients.

	HNE		MDA		Hb	
	r	p	r	p	r	p
Hb	-0.75	< 0.01	-0.81	< 0.01	–	–
LVMI	0.54	< 0.01	0.41	< 0.01	-0.65	< 0.01

The Standard Curve of HNE



Typical chromatogram

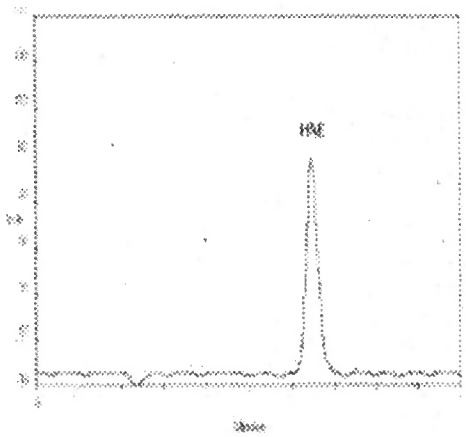


Fig. 1: Chromatogram of HNE in patients, control and standard.

DISCUSSION

Chronic renal insufficiency (CRI), once established, tends to progress to end-stage renal failure. Progression occurs even when the primary lesion has been treated or it is apparently inactive. The efforts to stop or even slow the progression of CRI have been likely unsuccessful[10].

It is well known that reactive oxygen species (ROS) play an important role in the pathogenesis and progression of renal failure. Independent of the renal disease itself and uremia, a lot of other factors contribute to oxidative stress[11] including haemodialysis via contact-activation of neutrophils through the artificial material of dialysis membranes[12] and disturbance in the enzyme system which protects against oxygen free radicals (OFRs) and it is believed that uremic patients may have a reduced antioxidative capacity and may be vulnerable to OFRs[13]. Previous research has shown that uremic patients, dialyzed or not, present serious disturbances in the status of trace elements especially those involved in the antioxidant system i.e. copper, zinc and selenium[14]. We studied oxidative stress in children with chronic renal failure (CRF) through estimating lipid peroxidation products (LPO), hydroxynonenal (HNE) & malodialdhyde (MDA), and interpreted them with the degree of anemia and left ventricular hypertrophy (LVH). There was a significant increase in systolic and diastolic blood pressure in patient groups compared with the healthy control; ($p < 0.01$), although the prevalence of hypertension in group I (65%) and in group II (75%) showed a non-significant difference; ($p > 0.05$). Richard et al.[15], reported that hypertension is an important complication that affects the overall prognosis of patients with ESRD. Regarding hemoglobin level, there was a significant difference among the 3 studied groups; ($p < 0.05$). As renal function decreased, a slow progressive decrease in hemoglobin concentration occurs, particularly becoming evident when glomerular filtration rate falls below 30 ml/minute[16]. Applying the LSD test between patient groups as regards their hemoglobin levels showed a non-significant increase of the mean hemoglobin level in group II (dialyzed patients treated with erythropoietin); ($p > 0.05$). This may be explained by the fact that erythropoietin alone is not effective in treatment of anemia in patients with chronic renal failure and other factors as iron must be supplied and dialysis must be regular and effective. Eckardt[17] provided similar results and explanation.

The results of this study demonstrated that lipid peroxidation products, HNE and MDA, both are increased in patients with chronic renal failure (dialyzed and non-dialyzed) than the healthy control; ($p < 0.01$). These results may point to increased lipid peroxidation that indicates oxidative stress in patients with CRF. Similar results were reported by Durak et al.[18] and Seims et al.[19] who reported that the CRF patients have a higher oxidative stress than normal subjects, but dialysis has a good effect in decreasing the oxidative stress.

Our study also showed that HNE was significantly lower in group II (dialyzed) than group I (non-dialyzed); $p < 0.05$. This may be due to the beneficial effect of dialysis on oxidative stress. However,

Hutqvist et al.[20], found that there is no difference in the levels of MDA between hemodialyzed patients and normal subjects. The non-significant difference in MDA between the patient groups may be due to lower sensitivity of MDA as an indicator of LPO than HNE[6]. Dasgupta et al.[21] showed less lipid peroxidation products when a more biocompatible polysulfone dialyzing membrane and vitamin E coated membrane is used. This further supports the concept that the dialysis process itself and the type of membrane play a role in the generation of free radicals.

In this study, we found a relation between lipid peroxidation and the state of anemia in patient groups, where there was a significant negative correlation between Hb concentration and lipid peroxidation product levels (HNE & MDA); ($r = -0.75$ & $r = -0.81$ respectively). In group I (non-dialyzed), patients with Hb < 9.5 gm/dl had significantly higher levels of HNE & MDA than those with Hb > 9.5 gm/dl; $p < 0.01$. Similar results were obtained in group II (dialyzed); $p < 0.01$. These results may be explained by the fact that anemic patients have limited oxygen transport capacity increasing anaerobic metabolism due to hypoxemia and ischemia and leading to increased blood concentration of free radical generators as catecholamine metabolizing enzyme, etc.[22]. On the other hand, RBC deficiency is accompanied by a deficit of reduced glutathione and of enzymes able to metabolize aldehydic LPO products. Thus the blood of uremic patients loses a major part of its antioxidant power[23] so treatment with EPO improves anemia and thus decreases the LPO products. Similar results were obtained by Sommerburg et al.[24], who reported that HNE and MDA decrease with treatment of anemia by EPO and under dialysis. Siems et al.[19] also documented that lipid peroxidation products decrease by increase of hemoglobin (Hb) concentration.

LVH is an independent factor of cardio-vascular-specific mortality in patients with CRF[25]. Left ventricular mass index (LVMI) showed a highly significant increase in CRF patients (dialyzed & non-dialyzed) than control; $p < 0.001$. However, LVMI in group II (dialyzed) was significantly higher than that in group I (non-dialyzed); $p < 0.01$. These data mean that left ventricular hypertrophy (LVH) was predominant in CRF patients with more predominance of LVH in group II (dialyzed). The prevalence of LVH in our study was 65% in group I (non-dialyzed) and 80% in group II (dialyzed). LVMI was significantly higher in CRF patients with Hb levels < 9.5 gm/dl than in those patients with Hb levels > 9.5 gm /dl; $p < 0.01$, in both group I and group II. A significant negative correlation between Hb levels and LVMI was found in patient groups; ($r = -0.65$, $p < 0.01$).

Our study also showed a positive correlation between lipid peroxidation products (HNE & MDA) and LVMI; $r = 0.054$ & 0.041 respectively. Scharer et al.[26], reported increased LVMI in children with CRF and after renal transplantation. Also, in a study performed by the European Dialysis and Transplant Association in children up to 15 years, LVH was found in 51% of patients on hemodialysis, in 20% on peritoneal dialysis and in 22% after renal

transplantation[27]. Our results coincide with Siems et al.[14], who reported that correction of anemia in CRF represents antioxidative therapy and decreases cardiovascular risks. Hampl et al.[28] reported that correction of anemia in CRF may lead to reduction of LVMI, may lead to cardiovascular benefit in hemodialysis and may also reduce oxidative stress. In conclusion, oxidative stress and lipid peroxidation represent a major health problem in CRF patients. Optimized correction of anemia as well as regular efficient dialysis help to reduce oxidative stress including lipid peroxidation, thereby reducing cardiovascular morbidity in patients with CRF, thus improving their quality of life and improving the prognosis of renal failure.

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