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Original Article

Plasma F_2-Isoprostane: A Biochemical Link Between Tissue Inflammation, Lipid Peroxidation and Cerebral Atherosclerosis in Uremic Children

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ABSTRACT

Background: Cerebral atherosclerosis is a major contributing factor to the high prevalence of cerebrovascular mortalities in uremic children. Enhancement of lipid peroxidation (LPO), attributable mainly to inflammation, is an important risk factor for premature atherosclerosis in chronic renal failure (CRF). Plasma F_2-isoprostane may provide a quantitative index of LPO, thus it could be a link between inflammation, LPO and atherosclerosis in CRF.

Objective: This study aimed at investigation of LPO (measured by plasma F_2-isoprostane) in relation to inflammation (indicated by C-reactive protein "CRP") and cerebral atherosclerosis, assessed by transcranial Doppler ultrasonography (TCD), in patients with CRF. In addition, the suppressive effect of oral vitamin E antioxidant therapy on LPO, measured by plasma F_2-isoprostane, was also investigated.

Methods: Thirty-four patients with CRF (22 on regular hemodialysis "HD" and 12 on conservative management) were studied in comparison to 34 healthy children serving as controls. Beside clinical evaluation, assessment of plasma F_2-isoprostane (ELISA), CRP and cerebral circulation using TCD was done. Vitamin E was supplied orally to patients with high plasma F_2-isoprostane levels (400 IU daily for two months) and re-estimation of plasma F_2-isoprostane was done after completion of therapy.

Results: Patients with CRF, either on regular HD or on conservative management, had significantly higher plasma F_2-isoprostane and CRP levels than healthy controls. Plasma F_2-isoprostane levels were elevated in 61.8% of CRF patients. There was a significant positive correlation between plasma F_2-isoprostane and CRP levels. Clinical neurological manifestations (such as transient ischemic attacks, convulsions, pyramidal weakness and peripheral neuritis) were found in 14 patients with CRF (41.2%). TCD abnormalities (impaired vasoreactivity "VR" ± flow abnormalities), which may point out to the presence of cerebral atherosclerosis, were found in 70.6% of CRF patients. These abnormalities were found in all patients with and in 50% of those without clinical neurological manifestations. There was a significant positive association between TCD abnormalities and both plasma F_2-isoprostane and CRP. In addition, vitamin E antioxidant therapy resulted in a significant decrease of plasma F_2-isoprostane levels.

Conclusion: LPO, measured by plasma F_2-isoprostane, may be enhanced in uremic patients. This may be attributable to the inflammatory process of the disease. Both LPO and inflammation may be important factors contributing to premature cerebral atherosclerosis, assessed by TCD, in these patients. In addition, vitamin E supplementation, as an antioxidant therapy, may result in suppression of the enhanced LPO in uremic patients. Wider scale studies investigating the suppressive effect of different regimens of vitamin E on LPO, measured by plasma F_2-isoprostane, are warranted as this LPO marker may serve as an indicator for the effectiveness of antioxidant strategies in patients with CRF.

INTRODUCTION

Patients with chronic renal failure (CRF) show a high prevalence of cerebrovascular disease which is a major

cause of their death. Atherosclerosis and related hypoperfusion can be considered as the paramount cause of cerebral damage[1,2] and enhancement of the incidence of stroke in CRF[3]. Cerebral atherosclerosis could be assessed by transcranial Doppler (TCD) ultrasonography which is a non-invasive useful technique for estimation and monitoring of cerebral circulation in uremic patients[4,5].

Increased lipid peroxidation (LPO) is a major risk factor for premature atherosclerosis in uremia[6,7]. In CRF, LPO is attributable mainly to inflammation resulting from the renal disease itself[8] and hemodialysis (HD) membrane bioincompatibility[9]. C-reactive protein (CRP) is one of the important inflammatory markers currently considered as a significant predictor of mortality in CRF[10].

Inflammation results in excessive production of free radicals which attack the polyunsaturated fatty acids constitutive of cellular membranes resulting in formation of end products of LPO[11]. These products are toxic to endothelium resulting in endothelial dysfunction which is the key initial event in the development of atherosclerosis due to the decreased production of the naturally occurring vasodilator nitric oxide[12]. F_2-isoprostane, a prostaglandin $F_2\alpha$-like compound biosynthesized from arachidonic acid nonenzymatically, has been shown to be a reliable biomarker of LPO[13]. It could be a biochemical link between inflammation, LPO and accelerated atherosclerosis in CRF[9].

In CRF, the activity of the non-enzymatic antioxidant defense (e.g., vitamin E, A and C) is reduced due to low dietary intake and/or removal by dialysis[14]. There is no doubt that the correction of the oxidant / antioxidant imbalance is an important approach for the reduction of the risk of cerebrovascular and cardiovascular diseases secondary to atherosclerosis in CRF patients[15]. Vitamin E is the most frequent anti-oxidant strategy in CRF patients[16]. F_2-isoprostane could be a useful indicator to the effectiveness of the interventions which decrease the oxidative stress and the associated inflammation[17].

AIM OF THE WORK

This study aimed at investigation of LPO (measured by plasma F_2-isoprostane) in relation to inflammation (indicated by C-reactive protein "CRP") and cerebral atherosclerosis, assessed by transcranial Doppler ultrasonography (TCD), in patients with CRF. In addition, the suppressive effect of oral vitamin E antioxidant therapy on LPO, measured by plasma F_2-isoprostane, was also investigated.

METHODS

Study population

This case-control, follow-up study was conducted in the Pediatric Dialysis Unit of Ain Shams University Hospitals, over a period of one year, from the beginning of March 2005 to the end of February 2006. It included 34 patients with CRF. They were 21 males and 13 females. Their ages ranged between 6 and 16 years [mean ± SD = 14.2 ± 2.1 years, median (IQR) = 15 (3) years]. Patients with CRF were categorized into 2 groups as follows:

Group I (Patients on regular hemodialysis):

It included 22 patients with end stage renal disease (ESRD) undergoing regular

hemodialysis (HD). They were 14 males and 8 females. Their ages ranged between 11 and 16 years [mean ± SD = 14.6 ± 1.6 years, median (IQR) = 15 (3) years]. HD was performed with carbonate dialysate for 2 to 4 hours in each session. All were on thrice HD regimen per week. Dialyzer surface area ranged from 0.7 to 0.9 m^2. All patients were on polysulfone type of dialyzer membrane.

Group II (Patients on conservative management):

It included 12 patients with CRF on conservative therapy. They were 7 males and 5 females. Their age ranged between 7 and 16 years [mean ± SD = 13.6 ± 2.8 years, median (IQR) = 14.5 (4) years].

Inclusion criteria: Stable, maintenance and regular HD patients with a duration of dialysis therapy of at least 2 years and a prognosis to survive for the duration of the study.

Exclusion criteria: Medical instability, therapy with agents that have been associated with elevated plasma lipids (e.g. beta-adrenergic blocking agents and androgens) and lipid lowering drugs, associated condition known to increase oxidative stress (e.g. diabetes mellitus and vascular connective tissue diseases) and presence of clinical evidence of infection.

Results of the patients were compared with 34 age- and sex-matched healthy children with no history of renal, neurological or any other medical problems serving as controls. They were 21 males and 13 females. Their ages ranged between 7 and 16 years [mean ± SD = 13.6 ± 2.7 years, median (IQR) = 14.5 (4.3) years].

An informed written consent for participation in the study was signed by the parents or the legal guardians of the studied subjects.

Study design

Clinical evaluation of the patients was done based on clinical history from the caregivers, reviewing follow-up sheets and clinical examination. Special emphasis was laid on disease duration, duration of dialysis, dialyzer type and surface area, drug therapy, manifestations suggesting nervous system involvement (such as convulsions, weakness, paraesthesia and transient ischemic attacks "TIAS") and measurement of blood pressure. In addition to routine laboratory investigations [blood hemoglobin % using coulter counting, blood urea using enzymatic rate method[18], serum creatinine using a modified rate Jaffe method[19] and serum albumin using concentration method[20] (Synchron CX5 system from BECKMAN, USA)], assessment of CRP (by latex agglutination), plasma F_2-isoprostane and cerebral haemodynamics using TCD was done. In addition, vitamin E was supplied orally to patients with elevated plasma F_2-isoprostane levels in the form of E-viton capsules (100 mg or 400 IU), from Cairo Pharmaceutical Company, as one capsule per day for 2 months. After completion of vitamin E antioxidant therapy, plasma F_2-isoprostane levels were re-estimated.

Study measurements

Sample collection

Six mL of venous blood were collected from each subject (before the dialysis session of patients on regular HD). One mL of blood dispensed gently into a tube containing EDTA as an anticoagulant for hemoglobin

assay. Three mL were transferred into a dry clean tube and left to clot and prompt separation of serum was done and used for direct assay of urea, creatinine, albumin and CRP. The other two mL were collected in a tube containing EDTA and after centrifugation, plasma was separated and stored at -80°C until assay of F_2-isoprostane. After completion of vitamin E therapy, two ml of venous blood were withdrawn from CRF patients for re-estimation of plasma F_2-isoprostane.

Determination of plasma F_2-isoprostane:

This assay is a competitive enzyme-linked assay (ELISA) using Bioxytech F_2-isoprostane immunoassay from Oxis international, USA. F_2-isoprostane in the samples or standards competes for the binding (to the antibody coated on the plate) with F_2-isoprostane conjugated to horseradish peroxidase (HRP). The peroxidase activity results in colour development in the substrate when added. The intensity of the colour is proportional to the amount of F_2-isoprostane bound and inversely proportionate to the amount of F_2-isoprostane in the samples or standards[21]. Patients with CRF were considered to have elevated plasma F_2-isoprostane if their levels were above 90.5 pg/ml which was the 95th percentile of the values of healthy controls as data distribution was non-parametric.

Estimation of cerebral circulation by TCD ultrasonography:

TCD was performed, before the dialysis session in patients on regular HD, using a pulsed Doppler device (DWL Electronische system, GmbH, Germany), operating at 2 MHz. The highest peak systolic and mean blood flow velocities in 2 mm increments in the middle, anterior and posterior cerebral arteries, internal carotid artery, vertebral and basilar arteries were recorded for each patient. CO_2 wash out by hyperventilation for 30 seconds was performed while insonating MCA vessel. Patients were considered to have positive TCD findings of large cerebral vasculature if they have maximum flow velocity greater than 200 cm/s, maximum velocity in the posterior cerebral, vertebral or basilar arteries greater than the maximum velocity in the middle cerebral artery, mean blood flow velocity of more than 120 cm/sec. and turbulence, decreased flow velocity in the segment distal to the stenotic lesion, interside difference of more than 25%, spectra broadening and arterial wall covibration[22]. Also, a drop in flow velocities less than 35% with hyperventilation denoted impaired vasoreactivity (VR) of medium sized and small arterioles[23].

Statistical Analysis:

The results were analyzed by commercially available software package (Stat View, Abacus Concepts, Inc, Berkley, CA, USA). The data were presented as mean and standard deviation (SD) in addition to median and interquartile range (IQR) which is the difference between the 75th and 25th percentiles. Mann Whitney test was used for comparison between 2 groups as data distribution was non-parametric. Wilcoxon signed rank test was used for comparison between the same groups before and after vitamin E supplementation. Spearman's correlation coefficient "r" was used to determine the relationship between different quantitative variables. For all tests, a probability (p) of less than 0.05 was considered significant.

RESULTS

Lipid peroxidation (measured by plasma F_2-isoprostane) and tissue inflammation (measured by CRP) in CRF:

Patients with CRF, either on regular HD or on conservative management, had significantly higher plasma F_2-isoprostane and CRP levels than healthy controls. Although, patients on regular HD had higher plasma F_2-isoprostane and CRP levels than those on conservative management, these differences did not reach statistical significance (Table 1).

Elevated plasma F_2-isoprostane levels were found in 61.8%, 72.7% and 41.7% of all CRF patients, patients on regular HD and those on conservative management, respectively. On the other hand, CRP positivity was found in 58.8%, 68.2% and 41.7% of all CRF patients, patients on regular HD and those on conservative management, respectively.

Plasma F_2-isoprostane had a significant negative correlation with blood hemoglobin ($r = -0.96$, $p < 0.001$) and serum albumin ($r = -0.87$, $p < 0.001$) and significant positive correlation with blood urea ($r = 0.6$, $p < 0.001$) and serum creatinine ($r = 0.9$, $p < 0.001$) among patients with CRF.

Relationship between plasma F_2-isoprostane, as an index of lipid peroxidation, and CRP, as an index of tissue inflammation, in CRF:

There was a significant positive correlation between plasma F_2-isoprostane and CRP levels ($r = 0.9$, $p < 0.001$). Also, there was a significant positive association between plasma F_2-isoprostane and CRP as 18 out of the 21 patients with elevated plasma F_2-isoprostane (85.7%) had positive CRP as well. On the other hand, 11 out of the 13 patients with normal plasma F_2-isoprostane (84.6%) had also negative results for CRP ($X^2 = 14.4$, $p < 0.001$). In addition, CRF patients with elevated F_2-isoprostane levels had significantly higher CRP values [mean ± SD = 55.8 ± 53.5, median (IQR) = 47 (48) mg/L] than patients with normal plasma F_2-isoprostane levels [mean ± SD = 4.5 ± 3.6, median (IQR) = 4 (2) mg/L, $z = 2.83$, $p < 0.001$]. Furthermore, CRF patients with positive CRP had significantly higher plasma F_2-isoprostane levels [mean ± SD = 45 ± 70.1, median (IQR) 122 (65.5) pg/mL] than those with negative CRP [mean ± SD = 70.9 ± 19, median (IQR) 69 (31) pg/mL, $z = 4.4$, $p < 0.001$].

TCD results of patients with CRF

TCD abnormalities were found in 24 out of the 34 studied CRF patients (70.6%). All of them had impaired VR, 7 had also generalized decrease of blood flow suggesting atherosclerosis and 2 had stenotic lesions of some large cerebral vessels as well.

Patients on regular HD had significantly lower MFV of the studied large cerebral vessels and VR than healthy controls (Table 2). On the other hand, VR of patients on conservative management [mean ± SD = 29.8 ± 19.2, median (IQR) = 33 (34.5) %] was significantly lower than controls [mean ± SD = 45.9 ± 3.1, median (IQR) = 47 (4) %, $z = 2.1$, $p < 0.05$]. In contrast, MFV of large cerebral vessels of both groups were comparable ($p > 0.05$).

Although TCD abnormalities (flow abnormalities and/or impaired VR) were higher in patients on regular HD (18/22 :

81.85%) than patients on conservative management (6/12 : 50%), this difference did not reach statistical significance ($p > 0.05$).

Relationship between TCD abnormalities and both oxidative stress, measured by plasma F_2-isoprostane, and tissue inflammation, measured by CRP, in CRF:

Patients with abnormal TCD had significantly higher plasma F_2-isoprostane and CRP than those with normal TCD (Table 3). Also, there was a significant positive association between TCD abnormalities and both plasma F_2-isoprostane ($X^2 = 22.9$, $p < 0.00001$) and CRP ($X^2 = 10.46$, $p < 0.01$) as 21 and 19 out of the 24 patients with abnormal TCD (85.5% and 79.2%, respectively) had also elevated plasma F_2-isoprostane and positive CRP, respectively. In addition, all and 8 out of the 10 patients with normal TCD had normal plasma F_2-isoprostane and negative CRP, respectively as well. Furthermore, there was a significant negative correlation between TCDVR and both F_2-isoprostane ($r = -0.57$, $p < 0.05$) and CRP ($r = -0.43$, $p < 0.05$).

Relationship between important clinical manifestations (neurological findings and hypertension) and results of plasma F_2-isoprostane, CRP and TCD in CRF patients:

Clinical neurological manifestations (such as TIAS, convulsions, pyramidal weakness and peripheral neuritis) were found in 41.2%, 50%, 25% of CRF patients compiled in one group, patients on regular HD and those on conservative management, respectively. CRF patients with neurological manifestations had significantly higher values of plasma F_2-isoprostane and CRP and lower TCD VR than those without such manifestations (Table 4). Interestingly all patients with neurological manifestations had TCD abnormalities. This frequency was significantly higher than that of patients without such manifestations (50%) ($X^2 = 9.9$, $p < 0.05$).

Fifteen out of the studied 34 CRF patients (44.1%) were hypertensive (12 on regular HD and 3 on conservative management). Hypertensive patients with CRF had significantly higher values of plasma F_2-isoprostane and CRP and lower TCD VR than those without hypertension (Table 4).

Effect of vitamin E supplementation on plasma F_2-isoprostane levels:

Vitamin E oral supplementation (400 IU daily for 2 months) resulted in a significant decrease of plasma F_2-isoprostane (Fig. 1). This antioxidant therapy resulted in normalization of plasma F_2-isoprostane in 16 out of the 21 patients with previously elevated plasma F_2-isoprostane (76.2%). In contrast, this antioxidant therapy had no significant effect in the remaining 5 patients (23.8%).

Table 1: Comparison between different studied groups in plasma levels of F_2-isoprostane and CRP.

Parameter	Patients on regular HD (n = 22)		Patients on conservative management (n = 12)		Controls (n = 34)		z1 (p)	z2 (p)	z3 (p)
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)			
Plasma F_2-isoprostane (pg/ml)	130.1 ± 73.6	107.5 (50.5)	92 ± 44.3	85 (35)	49.4 ± 18.7	45 (18)	5.7 (< 0.00001)**	3.5 (< 0.00001)**	1.8 (> 0.05)
CRP (mg/L)	45.6 ± 55.1	35.5 (44)	18.5 ± 28.2	4 (22)	1.6 ± 1.8	2 (2.5)	5.4 (< 0.001)**	3.3 (< 0.01)**	1.8 (> 0.05)

CRF: chronic renal failure; HD: hemodialysis, CRP: C-reactive protein, z1: comparison between patients on regular HD and controls; z2: comparison between patients on conservative management and controls; z3: comparison between patients on regular HD and those on conservative management.
$p > 0.05$: non-significant, $p < 0.01$, < 0.001, < 0.00001**: highly significant.

Table 2: Comparison between CRF patients on regular HD and controls in mean flow velocity and vasoreactivity of TCD.

	CRF patients on hemodialysis (n = 34)		Controls (n = 34)		z	p
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)		
MCAL (cm/second)	82.1 ± 25	91 (51)	110.5 ± 15.1	117 (21)	4.5	< 0.00001**
MCAR (cm/second)	82.3 ± 29.2	84 (63)	100 ± 9	99.5 (6.3)	2.4	< 0.05*
ACAL (cm/second)	68.1 ± 21.3	66 (45)	87.1 ± 12.4	90 (26.3)	3.5	< 0.00001**
ACAR (cm/second)	61.8 ± 24.2	61 (32.5)	81.5 ± 9.6	79.5 (16.3)	4	< 0.00001**
BA (cm/second)	62.9 ± 22.4	67.5 (32.5)	75.8 ± 10.8	73 (4)	2	< 0.05*
VR (%)	21.9 ± 14.2	22.5 (13.8)	45.9 ± 3.1	47 (4)	4.7	< 0.00001**

MCA: middle cerebral artery, ACA: anterior cerebral artery, BA: basilar artery, R: right, L: left, VR: vasoreactivity.
$p < 0.05$*: significant; $p < 0.00001$**: highly significant

Table 3: Comparison between plasma F_2-isoprostane and CRP of patients with and without TCD abnormalities.

	Patients with abnormal TCD		Patients with normal TCD		z	p
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)		
F_2-isoprostane (pg/ml)	116.7 ± 66.7	101 (59.8)	57.8 ± 37.8	44.5 (74.5)	4	< 0.00001**
CRP (mg/L)	70.9 ± 19	69 (31)	36 ± 48.7	18 (44)	2.8	< 0.01**

CRP: C-reactive protein, p < 0.01, 0.00001**: highly significant.

Table 4: Comparison between CRF patients with and without important clinical manifestations (neurological findings and hypertension) in plasma levels of F_2-isoprostane, CRP and TCD VR.

	F_2- isoprostane (pg/ml)			CRP (mg/L)			TCDVR (%)		
	Mean ± SD	Median (IQR)	z (p)	Mean ± SD	Median (IQR)	z (p)	Mean ± SD	Median (IQR)	z . (p)
Patients with neurological manifesta-tions (n = 14) Vs Patients without neurological manifesta-tions (n = 20)	157 ± 80.3 Vs 88.4 ± 35.6	131 (91.2) Vs 81.5 (40.7)	3.5 (< 0.00001)**	65.5 ± 60.1 Vs 15.4 ± 23.6	48 (72) Vs 4 (18.5)	3.4 (< 0.00001)**	15.9 ± 8.1 Vs 30.8 ± 17.9	17.5 (16) Vs 33 (33.5)	2.4 (< 0.05)*
Patients with hypertension (n = 15) Vs Patients without hypertension (n = 19)	144.1 ± 58.3 Vs 95 ± 66.2	127 (90) Vs 77 (41)	3.5 (< 0.00001)**	57.8 ± 47.4 Vs 18.8 ± 43.5	48 (72) Vs 4 (10)	3.3 (< 0.01)**	13.7 ± 9 Vs 33.3 ± 15.7	16 (19) Vs 40 (27)	3.3 (< 0.01)**

CRP: C-reactive protein, p < 0.05*: significant; < 0.01, 0.00001**: highly significant

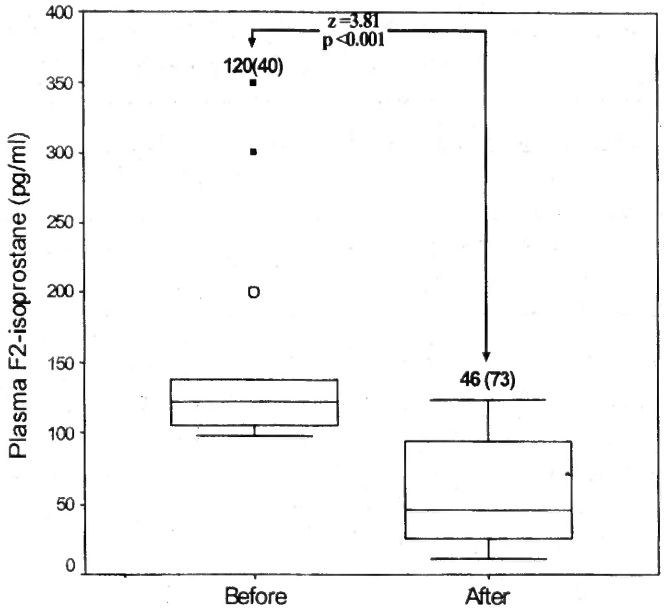

Fig. 1: Plasma F_2-isoprostane in CRF patients before and after oral vitamin E supplementation.

The boxes enclose the interquartile ranges (IQR) which are between the 25th and the 75th percentiles. The horizontal line inside the box represents the median and the whiskers represent the non outlier or extreme maximum and minimum values. The small open squares represent the outlier values (between 1.5 and 3 IQR). The small black squares indicate the extreme values which are more than 3 IQR.

DISCUSSION

Increased LPO, the central feature of oxidative stress, is attributable mainly to inflammation in CRF. Measurement of plasma F_2-isoprostane may provide a quantitative index of LPO and thus, oxidative stress in those patients[24]. LPO is an important risk factor for premature atherosclerosis[6,7] which is the major contributing factor to the high prevalence of cerebrovascular mortalities in uremic patients[25].

The present study revealed an increase of LPO in patients with ESRD (either on HD or conservative management), as determined by circulating total plasma F_2-isoprostane levels, when compared to healthy controls. Other studies also demonstrate high plasma levels of F_2-isoprostane in patients with CRF[9,10,24]. Similar to our results a previous study conducted on children with CRF[26] found a significant positive correlation between the plasma levels of F_2-isoprostane and the severity of CRF measured by blood urea and serum creatinine. In 2001, Handelman and colleagues[17] reported that increased plasma levels of F_2-isoprostane is the best evidence of in vivo oxidative stress in patients with ESRD.

CRF is a pro-oxidant state with an associated defect of the antioxidant defense resulting in imbalance between pro- and antioxidant systems[27]. However, the pathogenesis of oxidative stress in CRF patients remains poorly defined[11]. The suggested reasons for increased pro-oxidant state and hence LPO in CRF; include inflammatory process of the renal disease itself[28,29] and hemodialysis membrane bioincompatibility[9,10]. In our series,

although patients on regular HD had higher levels and frequency of elevated plasma F_2-isoprostane than those on conservative management, these differences did not reach statistical significance. In 2005, Marjani[30] reported increased LPO, measured by plasma malondialdehyde, in uremic patients after hemodialysis as compared to predialysis levels. The reason behind the non-significant difference in plasma F_2-isoprostane levels of patients on regular HD and those on conservative management in our study may be attributable to the use of a biocompatible type of hemodialyzing membrane. However, further studies concerning the effect of different types of hemodialysis membranes on plasma F_2-isoprostane levels are warranted to choose the biocompatible ones.

Inflammation was suggested by increased CRP levels in the serum of our studied uremic patients, without clinical evidence of infection, as compared to controls. Other investigators also reported increased CRP levels in uremic patients[8,24]. CRP is one of the important inflammatory markers currently considered as a significant predictor of mortality in uremia. CRF patients with high CRP (> 16.8 mg/L) levels were more than twice as likely to die as patients with low CRP levels[10].

An important clue for the etiopathogenic role of inflammation in LPO is our finding of a significant increase of plasma F_2-isoprostane in uremic patients with positive than those with negative CRP. Similar to our results, two previous studies reported significant positive association[9] and correlation[10] between plasma F_2-isoprostane and CRP. Thus, the relationship between both tissue inflammation (measured by CRP) and LPO (measured by F_2-isoprostane) may be a causal one in which inflammation may not only result in LPO but the intensity of the former determines the severity of the latter.

Another important factor contributing to enhanced LPO in CRF is anemia. RBCS deficiency is accompanied by reduced glutathione and enzymes capable to metabolize aldehydic LPO products[28]. This assumption was supported by our finding of a significant negative correlation between plasma F_2-isoprostane and blood hemoglobin levels. For this reason complete correction of renal anemia represents an effective mean of strengthening the antioxidant capacity and reduction of LPO[28].

The role of hypertension in causing increased oxidative stress in CRF is unknown[31]. In the present work, plasma F_2-isoprostane levels were significantly higher in hypertensive than normotensive CRF patients. Thus, hypertension may be another factor contributing to enhanced LPO in CRF. Wider scale studies are needed to prove this argument.

Previous investigators[6,7] reported that end products of LPO (e.g., F_2-isorpsotane) are toxic to endothelium resulting in endothelial dysfunction which is the key initial event in the development of atherosclerosis in CRF. For this reason, uremic patients have a high prevalence of cerebrovascular disease and strokes which are major causes of their death[1]. Thus, the increased levels of plasma F_2-isoprostane in patients with than those without clinical neurological manifestations could be attributable to the increased frequency of

atherosclerosis in the former than the latter groups. This premature atherosclerosis may be responsible for their cerebrovascular morbidity.

Cerebral blood flow studies using TCD is a non-invasive technique that measures blood flow velocity in large intracranial arteries. It is relatively cheap, can be performed with portal machines and allows monitoring for prolonged periods[4]. In the present study, MFV of large cerebral arteries of patients on regular HD were significantly lower than that of healthy controls and patients on conservative management. Similarly, two previous studies[5,32] demonstrated significantly lower MFV of patients under regular HD than those on conservative management. Besides atherosclerosis, the underlying process of the renal disease (anaemia and hypertension) in uremia could result in cerebrovascular insufficiency leading to brain damage and structural lesion[2]. Also, treatment specific changes in HD including hemoconcentration (due to fluid removal), alteration of hemostasis due to changes of plasma fibrinogen levels and endothelial activation could potentially interfere with cerebral blood flow[3]. Our series revealed a significantly lower TCD VR of uremic patients than healthy controls. Impaired VR may be attributable to arteriosclerosis. We could not trace data in literature regarding VR of cerebral circulation estimated by TCD in uremic patients to be compared with our results.

The possible valuable role of TCD in diagnosis of cerebral vascular abnormalities in CRF may be supported by our finding of a significant increase of TCD abnormalities in CRF patients with clinical neurological manifestations (100%) than those without such manifestations (50%). Moreover, the presence of TCD abnormalities in half of our patients without evident clinical neurological manifestations may highlight the importance of TCD evaluation of cerebral circulation in all uremic patients, even in absence of overt clinical neurological findings, for early interference before development of a debilitating neurological disease. It has recently been demonstrated that TCD can be used to detect circulating clinically silent cerebral emboli that can not be detected by any other currently available imaging modality by using a specific software. TCD microemboli signals (MES) are predictive of future stroke[4,33]. So, further studies assessing the frequency of TCD MES in uremic patients are warranted.

In our series, patients with abnormal TCD had significantly higher plasma F_2-isoprostane and CRP than those with normal TCD. Moreover, we found a significant positive association between TCD abnormalities and both of plasma F_2-isoprostane and CRP. The previous findings could be explained by enhancement of LPO secondary to the inflammatory process of uremia, both of which may result in premature cerebral atherosclerosis detected by TCD. Thus, F_2-isoprostane may be a biochemical link between, inflammation, LPO and accelerated atherosclerosis in uremic patients[9].

In 2006, Zwolinska and colleagues[14] reported low levels of vitamin E, A and C in CRF patients due to either low dietary intake and/or removal by dialysis. In the

present study, vitamin E (α-tocopherol) supplementation (400 IU/day for 2 months) resulted in a significant decrease of plasma F_2-isoprostane levels. We could not trace data in the literature regarding the effect of vitamin E supplementation on plasma F_2-isoprostane to be compared with to our results. Previous researchers reported the suppressive effect of vitamin E supplementation on other markers of LPO[34,35]. Vitamin E supplementation restores glomerular basement membrane integrity, prevents neutrophil chemotaxis and inhibits platelets aggregation[36].

In our series, vitamin E supplementation resulted in normalization of the elevated plasma F_2-isoprostane in 16 out of the 21 patients with elevated plasma F_2-isoprostane levels (76.2%). On the other hand, this antioxidant therapy had no significant effect in the remaining 5 patients (23.8%). Thus, further studies using different regimens of vitamin E supplementation to CRF patients with increased oxidative stress are mandatory to determine the best regimens (including dose and duration) of these therapeutic agents that result in complete suppression of oxidative stress in uremia. Synthetic biocompatible membranes, including vitamin E coated membranes, had antioxidant effects with preservation of plasma vitamin E levels in hemodialysis patients[37]. Thus, the use of these membranes is recommended to decrease the oxidative stress and hence the enhanced atherosclerosis responsible for cerebrovascular and cardiovascular mortalities in those patients.

In conclusion, LPO, measured by plasma F_2-isoprostane, may be enhanced in uremic patients. This may be attributable to the inflammatory process of the disease. Both LPO and inflammation may be important factors contributing to premature cerebral atherosclerosis, assessed by TCD, in these patients. The presence of TCD abnormalities in half of uremic patients without clinical neurological manifestations may highlight the importance of assessment of cerebral circulation in all CRF patients even in absence of overt clinical neurological findings. In addition, vitamin E supplementation, as an antioxidant therapy, may result in suppression of the enhanced LPO in uremic patients. Wider scale studies investigating the suppressive effect of different regimens of vitamin E on LPO, measured by plasma F_2-isoprostane, are warranted as this marker may serve as an indicator for the effectiveness of antioxidant strategies in patients with CRF. Moreover, studies concerning anti F_2-isoprostane measures as receptor antagonists or synthesis inhibition are needed as F_2-isoprostane could be a target for a new therapy which decrease LPO and hence, cerebrovascular mortalities from atherosclerosis in CRF.

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REFERENCES

1. **Savazzi, G.; Cusmano, F. and Musini, S. (2001):** Cerebral imaging changes in patients with chronic renal failure treated conservatively or in hemodialysis. Nephron.; 89 (1): 31-6.
2. **Skinner, H.; Mackaness, C.; Bedforth, N. and Mahajan, R. (2005):** Cerebral hemodynamics in patients with chronic renal failure: effects of hemodialysis. Br. J. of Anaesth.; 94 (2): 203-5.
3. **Stefanidis, I.; Bach, R.; Mertens, P.; Liakopoulos, V.; Liapi, G.; Munn, H.; et al. (2005):** Influence of hemodialysis on the mean flow velocity in the middle cerebral artery. Clin. Nephrol.; 64 (2): 129-37.
4. **Kron, J.; Hamper, U. and Petri, M. (2001):** Prevalence of cerebral microemboli in systemic lupus erythrematosus: transcranial Doppler. J. Rheumatol.; 28 (10): 2222-5.
5. **Kwiecinski, J.; Pierzchala, K.; Szczepanska, M. and Szprynger, K. (1998):** Doppler examination of cerebral arteries in uremic children. Pediatr. Nephrol.; 12 (9): 785-7.
6. **Caimi, G.; Carollo, C. and Lo Presti, R. (2004):** Chronic renal failure: oxidative stress, endothelial dysfunction and wine. Clin. Nephrol.; 62 (5): 331-5.
7. **Diepeveen, S.; Verhoeven, G.; Palen, J.; Dikkeschei, B.; Tits, L.; Kolsters, G.; et al. (2004):** Oxidative stress in patients with end-stage renal disease prior to the start of renal replacement therapy. Nephron. Clin. Pract.; 98: 3-7.
8. **Oberg, B.; McMenamin, E.; Lucas, F.; McMonagle, E.; Morrow, J.; Ikizler, T.; et al. (2004):** Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney Int.; 65 (3): 1009-16.
9. **Ikizler, T.; Morrow, J.; Roberts, L.; Evanson, J.; Becker, B.; Hakim, R.; et al. (2002):** Plasma F_2-isoprostane levels are elevated in chronic hemodialysis patients. Clin. Nephrol.; 58 (3): 190-7.
10. **Spittle, M.; Hoenich, N.; Handelman, G.; Adhikarla, R.; Homel, P. and Levin, N. (2001):** Oxidative stress and inflammation in hemodialysis patients. Am. J. kidney Dis.; 38 (6): 1408-13.
11. **Massy, Z. and Nguyen-Khoa, T. (2002):** Oxidative stress and chronic renal failure: markers and management. J. Nephrol.; 15: 336-41.
12. **Morris, S. and Jardine, A. (2000):** The vascular endothelium in chronic renal failure. J. Nephrol.; 13: 96-105.
13. **Basu, S. (2005):** Factors regulating isoprostane formation in vivo. Antioxidants and redox signaling; 7 (1-2): 221-35.
14. **Zwolinska, D.; Grzeszczak, W.; Szczepanska, M.; Killis-Pstrusinska, K. and Szprynger, K. (2006):** Vitamin A, E and C as non-enzymatic antioxidants and their relation to lipid peroxidation in children with chronic renal failure. Nephron. Clin. Pract.; 103 (1): 12-8.
15. **Neuzil, J.; Weber, C. and Kontush, A. (2001):** The role of vitamin E in atherogenesis: linking the chemical, biological and clinical aspects of the disease. Atherosclerosis; 157: 257-83.
16. **Meagher, E.; Barry, O.; Lawson, A.; Rokach, J. and Fitzgerald, G. (2001):** Effects of Vitamin E on lipid peroxidation in healthy persons. JAMA; 285: 1178- 82.
17. **Handelman, G.; Walter, M.; Adhikarla, R.; Gross, J.; Dallal, G.; Levin, N.; et al. (2001):** Elevated plasma F_2-isoprostanes in patients on long term haemodialysis. Kidney Int.; 59: 1960-6.
18. **Teitz, N. (editor) (1994):** Specimen collection and processing; sources of biological variations. In: Textbook of Clinical Chemistry, 2nd edition. W.B. Saunders. Philadelphia; p. 286.
19. **Vasiliades, J. (1976):** Determination of serum creatinine by a modified rate Jaffe method. Clin. Chem. Acta; 22: 1664.
20. **Doumas, B. and Peters, T. (1997):** Serum and urine albumin: a progress report on their measurement and clinical significance. Clin. Chem. Acta; 258 (1): 3-20.
21. **Morrow, J. and Roberts, L. (1996):** The isoprostanes. Current knowledge and directions for future research. Biochem. Pharmacol.; 51 (1): 1-9.
22. **Siebler, M.; Kleinschmid, A.; Sitzer, M.; Steinmetz, H. and Freund, J. (1994):** Cerebral micro-embolism in symptomatic and asymptomatic high-grade internal carotid artery stenosis. Neurology; 44: 615-8.
23. **Yokata, C.; Hasegawa, Y.; Minematsu, K. and Yamaguchi, T. (1998):** Effect of acetazolamide reactivity and long term outcome in patients with major cerebral artery occlusive diseases. Stroke; 29: 640-4.
24. **Lim, P.; Chang, Y.; Thien, L.; Wang, N.; Yang, C.; Chen, T.; et al. (2002):** 8-iso-prostaglandin F_2-α as a useful clinical biomarker of oxidative stress in ESRD patients. Blood Purif.; 20: 537-42.
25. **Carolio, T. and Presti, J. (2004):** Cerebrovascular abnormalities in uremic patients: A transcranial color-coded **sosngraphy** study. Am. J. Nephrol.; 38: 1128-68.
26. **Zwolinska, D.; Grzeszczak, W.; Kilis-Pstrusinska, K.; Szprynger, K. and Szczepanska, M. (2004):** Lipid peroxidation and antioxidant enzymes in children with chronic renal failure. Pediatr. Nephrol.; 19 (8): 888-92.
27. **Locatelli, F.; Canaud, B.; Eckardt, K.; Stenvinkel,**

P.; Wanner, C. and Zoccalic, C. (2003): Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol. Dial. Transplant.; 18: 1272-80.

28. **Siems, W.; Quast, S.; Carluccio, F.; Wiswedel, I.; Hirsch, D.; Augustin, W.; et al. (2002):** Oxidative stress in chronic renal failure as a cardiovascular risk factor. Clin. Nephrol.; 58 (1): S12-9.
29. **Kirschbaum, S. (2002):** Correlative studies of urine fluorescence and free radicals indicators. Clin. Nephrol.; 50 (5): 311-9.
30. **Marjani, A. (2005):** Effect of hemodialysis on plasma lipid peroxidation and erythrocyte antioxidant enzymes in Gorgan (**Souch** east of Caspian sea). Int. Nephrol.; 2 (1): 1105-20.
31. **Agarwal, R. (2004):** Chronic kidney disease is associated with oxidative stress independent of hypertension. Clin. Nephrol.; 61 (6): 377-83.
32. **Szprynger, K.; Kwiecinski, J.; Szczepanska, M. and Pierzchala, K. (2000):** Evaluation of cerebrovascular reactivity with chronic renal failure in children. Pediatr. Nephrol.; 14 (10-11): 993-6.
33. **Dahl, A.; Omdal, R.; Waterloo, K.; Joakimsen, O.; Jacobsen, E.; Koldingsnens, W.; et al. (2006):** Detection of cerebral emboli signals in patients with systemic lupus erythrematosus. J. Neurol. Neurosurg. Psychiatry; 77 (6): 774-9.
34. **Saran, R.; Novak, J.; Desai, A.; Abdulhayoglu, E.; Warren, J. and Bustami, R. (2003):** Impact of vitamin E on plasma asymmetric dimethylarginine (ADMA) in chronic kidney disease: a pilot study. Nephrol. Dial. Transplant.; 18: 2415-20.
35. **Roob, J.; Khoschsorur, G.; Tiran, A.; Horina, J.; Holzer, H. and Winklhofer-Roob, B. (2000):** Vitamin E attenuates oxidative stress induced by intravenous iron in patients on hemodialysis. J. Am. Soc. Nephrol.; 11: 539-49.
36. **Maccarrone, M.; Meloni, C.; Manca-di-Villahermosa, S.; Cococcetta, N.; Cascianic, U.; Finazzi-Agro, A.; et al. (2001):** Vitamin E suppressed 5-lipoxygenase-mediated oxidative stress mononuclear cells of hemodialysis patients regardless of administration route. Am. J. Kidney Dis.; 37: 964-9.
37. **Tamg, D.; Huang, T.; Liu, T.; Chen, H.; Sung, Y. and Wei, H. (2000):** Effect of vitamin E-bounded membrane on the 8-hydroxy 2-deoxyguanosine levels in leukocyte DNA of hemodialysis patients. Kidney Int.; 58: 90-9.