Plasma F2-Isoprostane: A Biomarker of Lipid Peroxidation: Correlation with Cerebral Haemodynamics in Children with Chronic Renal Failure

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ABSTRACT

Background: Increased lipid peroxidation (LPO), the central feature of oxidative stress, is attributable mainly to inflammation in chronic renal failure (CRF). Measurement of plasma F2-isoprostane may provide a quantitative index of LPO and thus, oxidative stress in those patients. LPO is an important risk factor for premature atherosclerosis which is the major contributing factor to the high prevalence of cerebrovascular mortalities and morbidities in uremic patients. Cerebral atherosclerosis and other flow abnormalities in CRF patients could be detected by Transcranial Doppler Ultrasonography (TCD). Vitamin E is the most frequent antioxidant strategy in CRF

Objective: This study aimed to investigate LPO (indicated by plasma F2-isoprostane levels), tissue inflammation (indicated by CRP levels) in patients with CRF in relation to cerebral atherosclerosis, estimated by TCD. In addition, the effect of oral vitamin E supplementation, as an antioxidant, on suppression of the enhanced LPO, measured by plasma F2-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 as controls. Beside full neurological evaluation, measurements of plasma F2-isoprostane (ELISA) and CRP and estimation of cerebral circulation by TCD were performed, with re-estimation of plasma F2 isoprostane after two months of Vitamin E supplementation for patients with high plasma F2-isoprostane.

Results: CRF patients, either complied in one group or subdivided into patients on regular HD and patients on conservative management, had significantly higher plasma F2-isoprostane and CRP levels than healthy controls. Plasma F2-isoprostane levels were elevated in 61.8%, 72.7% and 41.7% of all CRF patients, those patients on regular HD and patients on conservative management, respectively. There was a significant positive correlation between plasma F2-isoprostane and CRP levels. TCD abnormalities were found in 70.6% of CRF patients. There was significant positive correlation between TCD abnormalities and both plasma F2-isoprostane and CRP levels. Patients with clinical neurological manifestations (41.2%) had significantly higher levels of plasma F2-isoprostane and CRP and frequency of TCD abnormalities than those without such manifestations. Vitamin E supplementation resulted in a significant decrease of plasma F2-isoprostane levels.

Conclusion: LPO, measured by plasma F2-isoprostane, may be enhanced in uremic patients. This may be attributable to the inflammatory process, indicated by elevated CRP levels. Both LPO and inflammation may be important factors contributing to premature atherosclerosis in these patients. This assumption was suggested by the significant positive association between TCD abnormalities (70.6%), which point out to the presence of cerebral atherosclerosis, and both elevated plasma F2-isoprostane levels (61.8%) and CRP positivity (58.8%). In addition, vitamin E supplementation, as an antioxidant therapy, may result in suppression of the enhanced LPO in uremic patients indicated by a significant decrease of plasma levels of F2-isoprostane levels. But further studies on a large scale, using different regimens (doses and durations of therapy) of vitamin E, to choose the best regimen of this antioxidant therapeutic agent in CRF patients are warranted. Also, the effect of these agents on lowering plasma F2-isoprostane levels should be studied as this LPO marker may serve as an...

INTRODUCTION

Atherosclerotic vascular disease is a major cause of morbidity and mortality in patients with chronic renal failure. Oxidative stress, which occurs when there is excessive free radical production or low antioxidant levels, has emerged as an important cofactor for the development of endothelial dysfunction and hence, atherogenesis.

Increased lipid peroxidation (LPO), the central feature of oxidative stress, is attributable mainly to both the inflammatory process of the renal disease itself and hemodialysis (HD) membrane bio-incompatibility in patients with chronic renal failure (CRF). Both conditions result in excessive production of free radicals which attack the polyunsaturated fatty acids constitutive of cellular membranes resulting in formation of end products of LPO. Increased LPO, is a major risk factor for premature atherosclerosis in CRF.

Discovery of the F2-isoprostanes, a group of prostaglandin F2α-like compounds biosynthesized from arachidonic acid nonenzymatically, has uncovered a new and novel facet of free radical biology. F2-isoprostanes has been shown to be a reliable biomarker of lipid peroxidation. CRP is one of the important inflammatory markers currently considered as a cardiovascular risk factor and it is a significant predictor of mortality in CRF.

F2-isoprostane is toxic to endothelium resulting in endothelial dysfunction which is the key initial event in the development of atherosclerosis. This results from the decreased production of the naturally occurring vasodilator nitric oxide, leading to vasoconstriction. Uremic patients show a high prevalence of cerebrovascular disease which is also a major cause of their death. Besides atherosclerosis, the underlying process of renal disease (anemia and hypertension) and treatment specific changes in HD (hemoconcentration and alteration of hemostasis) could result in cerebrovascular insufficiency and increasing the incidence of stroke in these patients.

Transcranial Doppler (TCD) is a non-invasive technique that measures blood flow velocities in large intracranial arteries. TCD is a useful method of assessment of cerebral circulation in uremic patients.

In CRF the activity of the antioxidant defense (e.g., vitamin E, A and C) is reduced due to low dietary intake and/or removal by dialysis. Vitamin E is the most frequent anti-oxidant strategy in CRF patients. There is no doubt that the correction of the oxidant / antioxidant imbalance is an important approach for the reduction of the risk of cardiovascular and cerebrovascular diseases secondary to atherosclerosis in CRF patients.

Aim of the work

This study aimed at investigation of lipid peroxidation (indicated by plasma F2-isoprostane levels), tissue inflammation (indicated by CRP levels) in patients with CRF in relation to cerebral atherosclerosis, estimated by TCD. In addition, the effect of oral vitamin E supplementation, as an antioxidant, on suppression of the enhanced LPO, measured by plasma F2-isoprostane, was also investigated.

SUBJECTS AND METHODS

Study population

This case-control, follow-up study was conducted in the Pediatric Dialysis Unit and Neurology department of Ain Shams University Hospitals. It included 34 patients with CRF. An informed written consent for participation in the study was signed by the parents or the legal guardians. 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). HD was performed with carbonate dialysate for 2 to 4 hours in each session. All were on thrice HD regimen per week. All patients were on polysulfone type of dialyzer membrane.

- **Group II (Patients on conservative management)**
  It included 12 patients with CRF on conservative therapy.

Inclusion criteria:
Stable, maintenance and regular HD patients with duration of dialysis therapy for 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 presence of clinical evidence of infection.

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 manifestations suggesting nervous system involvement (as convulsions, weakness, paraesthesia, transient ischemic attacks "TIAs").

Group III (Control group)

It included 34 age- and sex-matched healthy children with no history of renal or other medical problems.

Study measurements

The following was done for all subjects:

a. Detailed history taking and thorough clinical examination

b. Laboratory investigations including:

1. Blood urea, using enzymatic rate method\(^1\), serum creatinine, using a modified rate Jaffe method\(^1,19\) (Synchron CX5 system from BECKMAN, USA).

2. C-reactive protein (CRP) by latex agglutination.

3. Plasma F2-isoprostane by competitive enzyme linked immunoassay (ELISA)\(^20\) using Bioxytech 8-isoprostane immunoassay from Oxis international, USA.

c. Transcranial Doppler ultrasonography “TCD”

d. Drug therapy: twenty one patients in whom plasma F2-isoprostane levels were elevated were given 400 IU of vitamin E in the form of E-viton capsules 100 mg (from Cairo Pharmaceutical Company) as one capsule per day for 2 months.

After completion of vitamin E-antioxidant therapy, re-estimation of plasma F2-isoprostane levels was done.

Sample collection

Three millimeters of venous blood were collected from each subject (after the dialysis session in patients on regular HD). Prompt separation of serum was carried out and used for direct assay of urea, creatinine and CRP. CRP was normal if less than 10 mg/ Liter\(^1\). Part of serum was stored at -20 °C until assay of F2-isoprostane.

Re-assay of F2-isoprostane was done after two months of vitamin E therapy for CRF patients with high plasma F2-isoprostane levels.

Determination of plasma F2-isoprostane by ELISA

Principle of F2-isoprostane assay:

This assay is a competitive enzyme-linked immunoassay (ELISA) for determining levels of F2-isoprostane in biological samples. Briefly, 8-epi-prostaglandin F2α (8-EPI) or (F2-isoprostane) in the samples or standards competes for the binding (to the antibody coated on the plate) with F2-isoprostane conjugated to horseradish peroxidase (HRP). The peroxidase activity results in colour development in the substrate when added. The intensity of the color is proportional to the amount of 8-EPI-HRP bound and inversely proportionate to the amount of 8-EPI in the samples or standards\(^21\).

Patients with CRF were considered to have elevated serum F2-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.

Transcranial Doppler

TCD was performed, after 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., turbulence, decreased flow velocity in the segment distal to the stenotic lesion, inter-side difference of more than 25%, spectral broadening and arterial wall co-vibration or generalized diminished mean flow velocities. Also, a drop in flow velocities less than 35% with hyperventilation denoted impaired vasoreactivity (VR) of arterioles.

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). Mann Whitney test was used for comparison between 2 groups as data distribution was non-parametric. 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

The study 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]. Patients were categorized as either patients on regular hemodialysis (group I, 22 patients; 14 males and 8 females with ages ranged between 11 and 16 years [mean±SD = 14.6±1.6 years]) and patients on conservative managements (group II, 12 patients; 7 males and 5 females with ages ranged between 7 and 16 years [mean±SD = 13.6±2.8 years]). Patients were compared to 34 age and sex matched healthy children in respect to F2-isoprostane, CRP and TCD findings.

Lipid peroxidation (measured by plasma F2-isoprostane) and tissue inflammation (measured by CRP) in CRF:

The mean values of plasma F2 isoprostane in all studied patients with CRF, patients on regular HD and patients on conservative management was 116.75±66.7, 130.1±73.6 and 92±44.3 pg/mL respectively and are significantly higher than that of healthy controls (49.4±18.7 pg/mL), (p<0.00001) (Table I). Although, patients on regular HD had higher plasma F2-isoprostane levels than those on conservative management, these differences did not reach statistical significance (p>0.05). Interestingly, 61.8% (21/34 patients), 72.7% (16/22 patients) and 41.7% (5/12 patients) of all CRF patients, those on regular HD and patients on conservative management had elevated plasma F2-isoprostane levels. CRP positivity was found in 58.8% (20 patients), 68.2% (15 patients) and 41.7% (5 patients) of all CRF patients, group I and group II, respectively. The mean values of CRP were 36±4.8, 55.1±5.6 and 28.2±5.6 mg/L in all CRF patients, group I and group II respectively, significantly higher than control group (1.8±1.6 mg/L) (p<0.001, p<0.001 and p<0.01 respectively) (Table 1). Yet, there is no statistical difference regarding CRP between group I and group II (p>0.05).

Relationship between plasma F2-isoprostane, as an index of lipid peroxidation and CRP, as an index of tissue inflammation in CRF:

There was a significant positive association between plasma F2 isoprostane and CRP. Eighteen out of the total 21 patients with elevated plasma F2-isoprostane (85.7%) had positive CRP as well. On the other hand, 11 out of the 13 patients with normal F2-isoprostane (84.6%) had also negative results for CRP (p<0.001).

Interestingly, CRF patients with elevated F2-isoprostane levels had significantly higher CRP values [mean±SD = 55.8±53.5 mg/L] than patients with normal plasma F2-isoprostane levels [mean±SD = 4.5±3.6 mg/L, p<0.001]. In addition, CRF patients with positive CRP had significantly higher plasma F2-isoprostane levels [mean±SD=70.9±19 pg/mL] than those with negative CRP [mean±SD = 45±7.01 pg/mL, p<0.001].

TCD results

TCD abnormalities were found in 24 out of the studied CRF patients (70.6%). All of them had impaired VR, 7 had also generalized decreased mean blood flow velocities i.e. atherosclerosis and one patient with left MCA stenosis and one patient with right ACA stenosis.
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 in group II [mean±SD = 29.8±19.2 %] was significantly lower than controls [mean±SD = 45.9±3.1%, p<0.05]. In contrast, MFV of large cerebral vessels of both groups (group I &II) 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 F2-isoprostane, and tissue inflammation measured by CRP in CRF:

Patients with abnormal TCD (24 patients, 70.6%) had significantly higher serum F2-isoprostane and CRP than those with normal TCD (Table 3).

There was significant positive correlation between TCD abnormalities and both elevated plasma F2-isoprostane (X² = 22.9, p < 0.00001) and CRP (X² = 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 F2-isoprostane and positive CRP, respectively. In addition, all and 8 out of the 10 patients with normal TCD had normal plasma F2 isoprostane and negative CRP, respectively as well (Table 3).

<table>
<thead>
<tr>
<th>Variable</th>
<th>All CRF patients N=34</th>
<th>Group I N=22</th>
<th>Group II N=12</th>
<th>Group III (Controls) N=34</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2—isoprostane (pg/mL) Mean±SD</td>
<td>116.75±66.7*</td>
<td>130.1±73.6**</td>
<td>92±44.3***</td>
<td>49.4±18.7</td>
</tr>
<tr>
<td>CRP (mg/L) Mean±SD</td>
<td>36±4.87*</td>
<td>55.1±45.6**</td>
<td>28.2±18.5***</td>
<td>1.8±1.6</td>
</tr>
</tbody>
</table>

* significant between all patients and groupIII P<0.00001  
# significant between all patients and groupIII P<0.001  
** significant between group I and III P<0.00001  
## significant between group I and III P<0.001  
***significant between group II and III P<0.00001  
###significant between group II and III P<0.01  
P < 0.000001:very Highly significant

Relationship between clinical neurological manifestations and Plasma F2 isoprostane, CRP and TCD in CRF patients:

Clinical neurological manifestations (as TIAs, convulsions, pyramidal weakness and peripheral neuritis) were found in 41.2%, 50% and 25% of CRF patients, group I, and group II, respectively. CRF patients with neurological manifestations had significantly higher values of plasma F2 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 findings (50%) (p<0.05).

Effect of vitamin E supplementation on plasma F2-isoprostane:

Vitamin E supplementation (400 IU daily for 2 months) resulted in a significant decrease of plasma F2-isoprostane (Fig. 1).

Vitamin E supplementation resulted in normalization of F2-isoprostane in 16 out of the 21 patients with elevated plasma F2-isoprostane (76.2%). In contrast, this antioxidant therapy had no significant effect in the remaining 5 patients (23.8%).

Correlation between plasma F2-isoprostane and the studied clinical, laboratory and cerebral hemodynamics (TCD) data of CRF patients:

Plasma F2-isoprostane had significant positive correlation with CRP (r = 0.9, p<0.001) and serum creatinine (r = 0.9, p<0.001) among patients with CRF and significant negative correlation with TCD VR (r = -0.57, p<0.05).
P < 0.001: Highly significant
P < 0.01: significant
CRF: Chronic renal failure; HD: Hemodialysis, CRP: C-reactive protein

Table 2. Comparison between Group I and Group III as regards mean flow velocities and vasoreactivity of TCD.

<table>
<thead>
<tr>
<th>variable</th>
<th>CRF patients on hemodialysis (N = 22) Mean±SD</th>
<th>Controls (n= 34) Mean±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCAL (cm/second)</td>
<td>82.1 ± 25</td>
<td>110.5 ± 15.1</td>
<td>(&lt; 0.00001)**</td>
</tr>
<tr>
<td>MCAR (cm/second)</td>
<td>82.3 ± 29.2</td>
<td>100 ± 9</td>
<td>(&lt; 0.05)*</td>
</tr>
<tr>
<td>ACAL (cm/second)</td>
<td>68.1+21.3</td>
<td>87.1 ± 12.4</td>
<td>(&lt; 0.00001)**</td>
</tr>
<tr>
<td>ACAR (cm/second)</td>
<td>61.8 ± 24.2</td>
<td>81.5 ± 9.6</td>
<td>(&lt; 0.00001)**</td>
</tr>
<tr>
<td>BA (cm/second)</td>
<td>62.9 ± 22.4</td>
<td>75.8 ± 10.8</td>
<td>(&lt; 0.05)*</td>
</tr>
<tr>
<td>VR (%)</td>
<td>21.9 ± 14.2</td>
<td>45.9 ±3.1</td>
<td>(&lt; 0.00001)**</td>
</tr>
</tbody>
</table>

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 F2-isoprostane and CRP of patients with and without TCD abnormalities.

<table>
<thead>
<tr>
<th></th>
<th>Patients with abnormal TCD (N=24) Mean±SD</th>
<th>Patients with normal TCD (N=10) Mean±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2-isoprostane (pg/ml)</td>
<td>116.7±66.7</td>
<td>57.8±37.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>70.9±19</td>
<td>36±48.7</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein, P<0.01, P<0.00001: highly significant.
Fig. (1): Effect of vitamin E supplementation on plasma F2-isoprostane in CRF patients with elevated plasma F2-isoprostane. (n=21). (The ranges are marked as maximum and minimum).

Table 4. Comparison between patients with and without clinical and neurological manifestations in plasma levels of F2-isoprostane, CRP and TCD VR.

<table>
<thead>
<tr>
<th></th>
<th>Patients with neurological manifestations (n=14)</th>
<th>Patients without neurological manifestations (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td></td>
</tr>
<tr>
<td>F2-isoprostane (pg/ml)</td>
<td>157±80.3</td>
<td>88.4±35.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.0001)**</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>56.5±60.1</td>
<td>15.4 ±23.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.0001)**</td>
</tr>
<tr>
<td>VR (%)</td>
<td>15.9±8.1</td>
<td>30.8±17.9</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.05*)</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein, VR: Vasoreactivity, p < 0.05*: significant, p < 0.00001**: highly significant

DISCUSSION

Increased LPO, the central feature of oxidative stress, is attributable mainly to inflammation in CRF. Measurement of plasma F2-isoprostane may provide a quantitative index of LPO and thus, oxidative stress in those patients. LPO is an important risk factor for premature atherosclerosis which is the major contributing factor to the high prevalence of cerebrovascular mortalities and morbidities in uremic patients. Cerebral atherosclerosis and other flow abnormalities in CRF patients could be detected by TCD.
The present study revealed increased LPO in patients with ESRD (on HD and conservative management), as determined by circulating total plasma F2-isoprostane levels, when compared to healthy controls. Other studies also demonstrate high plasma levels of F2-isoprostane in patients with CRF. Similar to the results of the study conducted by Zowlinska and colleagues, we found a significant positive correlation between the plasma levels of F2-isoprostane and the severity of CRF measured by blood urea and serum creatinine.

F2 isoprostane is a new group of stable prostaglandin (8-iso-prostaglandin 2alpha) PGF2α isomers formed by free radical peroxidation of arachidonic acid bound to the membrane phospholipids, independent of the action of cyclooxygenase (COX), 8-iso-PGF2α is the best characterized compound belonging to this group and it is a useful clinical index of oxidative stress. Isoprostanes being chemically stable end products of lipid peroxidation, are released and circulate as a free form or as esters in phospholipids in plasma. Also, plasma F2 isoprostane was found to be causally related to some acute phase reactant proteins such as CRP. For this reason, isoprostane has been recently considered an ideal marker for investigating the pathophysiology of oxidative injury. In 2001, Handelman and colleagues reported that increased plasma levels of F2 isoprostane is the best evidence of in vivo oxidative stress in patients with ESRD.

Oxidative stress results from imbalance between pro oxidant agents (reactive oxygen species ROS) and antioxidant systems. CRF is a pro-oxidant state with an associated defect of the antioxidant defense resulting in imbalance between pro-and antioxidant systems. However, the pathogenesis of oxidative stress in CRF patients remains poorly defined. The suggested reasons for increased pro-oxidant state and hence LPO in CRF: include inflammatory process of the renal disease itself and hemodialysis membrane bioincompatibility which is an additional inflammatory stimulus resulting in neutrophil and complement activation, with excessive ROS production which attack the lipid component of cellular membrane resulting in lipid peroxidation.

In our series, although patients on regular HD had higher levels and frequency of elevated plasma F2-isoprostane than those on conservative management, these differences did not reach statistical significance. Previous study reported increased LPO in CRF after hemodialysis as compared to predialysis levels. The reason behind the non-significant difference in plasma F2 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 F2-isoprostane levels are warranted to choose the biocompatible ones.

In this study, tissue inflammation was documented by increased CRP levels in uremic patients (with no clinical evidence of infection), either compiled as one group or subdivided into patients on regular HD and those on conservative management, as compared to controls. Other investigators also reported increased CRP levels in uremic patients.

CRP is one of the important inflammatory markers currently considered as a cardiovascular risk factor in uremia. Studies demonstrated that CRF patients with high CRP levels were more than twice as likely to die as patients with low CRP levels.

An important clue for the etiopathogenic role of inflammation in LPO is our finding of a significant increase of plasma F2-isoprostane in uremic patients who had positive CRP compared to those with negative CRP. Similar to our results, other studies reported a significant positive association between elevated plasma F2-isoprostane and CRP positivity. Thus, the relationship between both tissue inflammation (measured by CRP) and LPO (measured by F2-isoprostane) may be a causal one in which tissue inflammation not only result in LPO but the intensity of the former determines the severity of the latter. Therefore, similar to the results of the study conducted by Spittle and colleagues, our findings also demonstrate that CRF patients are exposed to both oxidative stress and inflammation. Thus, measurement of plasma F2-isoprostane may be a useful biomarker of oxidative stress status as well as in developing new therapeutic strategies to ameliorate inflammatory and oxidative injury in this patient population.
In the present work, CRF patients with clinical neurological manifestations had significantly higher plasma F2-isoprostane and CRP levels than those without such manifestations. This could be explained by the fact that increased LPO, due to tissue inflammation is a major risk factor for atherosclerosis in uremia. F2-isoprostane is toxic to endothelium resulting in endothelial dysfunction which is the key initial event in the development of atherosclerosis due to the reduced production of the vasodilator nitric oxide (NO) leading to vasoconstriction. For this reason, uremic patients have a high prevalence of cerebrovascular disease and strokes which are also major causes of their death.

Thus, the increased levels of both plasma F2-isoprostane and CRP in patients with than those without clinical neurological manifestations could be attributable to the increased frequency of atherosclerosis in the former than the latter group. This premature atherosclerosis (due to increased inflammation and LPO) may be responsible for the cerebrovascular morbidity of uremic patients.

Cerebral blood flow studies using TCD is a non-invasive technique that measures blood flow velocity in large intracranial arteries. It is relatively cheap, available and can be performed with portable machines.

In the present study, mean flow velocities of large cerebral arteries after dialysis management of patients on regular HD were significantly lower than that of healthy controls. Similarly, 2 previous studies 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 uremic could result in cerebrovascular insufficiency leading to brain damage and structural lesion. 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.

Our series revealed significant decrease of VR of uremic patients, either compiled as one group or subdivided into patients on regular HD and patients on conservative management, compared to healthy controls. Impaired VR, which may be attributable to atherosclerosis, occurred in 70.6%, 81.8% and 50% of all CRF patients, those on regular HD and those on conservative management, respectively. 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 variable 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%). 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 manifestations, for early interference before development of a debilitating neurological disease.

In our series, patients with abnormal TCD had significantly higher plasma F2-isoprostane and CRP than those with normal TCD. Furthermore, there was significant negative correlation between VR and both plasma F2-isoprostane and CRP levels. Moreover, we found a significant positive association between TCD abnormalities and both elevation of plasma F2-isoprostane and CRP positivity. All the previous findings may point out to the significant positive relationship between TCD abnormalities and both tissue inflammation and LPO in CRF.

The previous findings could be explained by enhancement of LPO secondary to the inflammatory process of uremia, both of which result in premature atherosclerosis with abnormalities of MFV and VR of the cerebral circulation detected by TCD. Thus, F2-isoprostane may be a biochemical link between, inflammation, LPO and accelerated atherosclerosis in uremic patients.

Studies demonstrated low levels of vitamin E, A and C in CRF patients due to either low dietary intake and/or removal by dialysis. Vitamin E is the most frequent antioxidant strategy in CRF patients. In the present study vitamin E (α-tocopherol) supplementation (400 IU/day for 2 months) resulted in a significant decrease of the elevated plasma F2-isoprostane.

The benefit of antioxidant therapy in CRF may result from the interruption of several
pathophysiological mechanisms. Vitamin E restores glomerular basement membrane integrity, prevents neutrophil chemotaxis and inhibits platelet aggregation.

In our series, vitamin E supplementation resulted in normalization of the elevated plasma F2-isoprostane in 16 out of the 21 patients with elevated plasma F2-isoprostane levels (76.2%). On the other hand, this antioxidant therapy had no significant effect in the remaining 5 patients (23.8%) and plasma F2-isoprostane levels remained high. Thus, further studies using different regimens of vitamin E and other antioxidants supplementation in 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.

Today there is no doubt that the correction of the oxidant / antioxidant imbalance in patients with CRF is an important approach for the reduction of the risk of those patients to develop cardiovascular disorders.

Synthetic biocompatible membranes, including vitamin E coated membranes, had antioxidant effects and preservation of plasma vitamin E levels in hemodialysis patients. Thus, the use of vitamin E coated membranes is recommended in CRF patient on regular HD to decrease the oxidative stress and hence, the enhanced atherosclerosis responsible for the cardiovascular and cerebrovascular mortalities in those patients.

In conclusion, both LPO, measured by plasma F2 isoprostane, and CRP may be enhanced in uremic patients. Both LPO and inflammation may be important factors contributing to premature atherosclerosis in these patients. The latter assumption was suggested by the significant positive association between TCD abnormalities (70.6%), which point out to the presence of cerebral atherosclerosis, and both elevated plasma F2 isoprostane levels (61.8%) and CRP positivity (58.8%).

In addition, vitamin E supplementation, as an antioxidant therapy, may result in suppression of the enhanced LPO in uremic patients indicated by a significant decrease of plasma levels of F2 isoprostane levels. But further studies on a large scale, using different regimens (doses and durations of therapy) of vitamin E, to choose the best regimen of this antioxidant therapeutic agent in CRF patients are warranted. Also, the effect of these agents on lowering plasma F2-isoprostane levels should be studied as this LPO marker may serve as an indicator for the effectiveness of antioxidant strategies in patients with CRF. In addition, F2 isoprostane, CRP and TCD could represent useful approach not only to monitor antioxidant treatment and new dialysis therapies, but also to monitor for occurrence of cerebral atherosclerosis. Studies concerning anti F2-isoprostane measures as receptor antagonists or synthesis inhibition are needed. As F2-isoprostane could be a target for a new therapy which decrease LPO and hence, cerebrovascular mortalities from atherosclerosis in CRF in future.

REFERENCES

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استخدام إف -2 أيزوبروستين كدلالة على تأكد الدهون وعلاقته بديناميكية الأوعية الدموية المخية في مرضى الفشل الكلوي الأطفال

تلعب أكسدة الدهون وما ينتج عنها من جزيئات الشوارد الحرجة، فيما يبدو، دوراً هاماً في تطور التلف الناتج عن العديد من الأمراض مثل الفشل الكلوي، وقد تحدث جزيئات الأكسجين المفقمة آثارها الضرار خلال تفاعليها مع البروتينات الخلوية والدهون والحمض النووي وتدمرها لكل منها.

وبعد إف -2 أيزوبروستين واحدا من مخرجات أكسدة الدهون التي لها دورها وعلاقتها بانحلال التهاب الأنسجة مثل بروتينات التفاعل وخلايا قد يكون من العوامل المسببة لتشيكل الشرايين، ويعاني مرضى الفشل الكلوي من تصلب الشرايين المبكر مما يكون له الأثر في مآل المرض.

وتهدف هذه الدراسة لدراسة إذا ما كانت تآكل علاج ما بين مستوى إف -2 أيزوبروستين في البلازما كدلالة على تأكد الدهون وما يسبب تغييرات في الدورة الدموية المخية.

تم قياس نسبة إف -2 أيزوبروستين في البلازما ونوعها الاصطناعية لتشريين الدم عبر الدماغ لعدد 34 مريض بالفشل الكلوي ومقارنته بنظرائهم من الأصحاء.

المتطلب:

بالمقارنة بين مستوى إف -2 أيزوبروستين وجد أنها ذات دلالة إحصائية أكبر في كافة المرضى عن نظرائهم من الأصحاء. كما أن ارتفاع مستوى تأكد الدهون في المرضى المصابون بتغيرات في الدورة الدموية المخية يشير إلى حدوث تصلب شرايين.

الاستنتاج:

ترجح هذه النتائج أن ارتفاع مستوى إف -2 أيزوبروستين في البلازما يعد كدالة على حدوث تأكد الدهون وهو عامل خطورته قد يتسبب في حدوث تصلب الشرايين كما أظهرتها الدراسات الصوتية لتشريين الدم عبر الدماغ.