FLUORIDE AGGRAVATION OF OXIDATIVE STRESS IN PATIENTS WITH CHRONIC RENAL FAILURE

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SUMMARY: Based on evidence that fluoride ion (F) increases the production of reactive oxygen species, inhibits antioxidant enzyme activity, and enhances lipid peroxidation, a study of these effects was conducted on 52 patients with chronic renal failure (CRF), of whom 33 were undergoing chronic haemodialysis (HD) with the use of polysulphone membrane dialysers, while 19 with less advanced CRF, who were not undergoing HD, were treated conservatively with angiotensin-converting enzyme inhibitors and diuretics. Serum concentrations of F, Cu, Zn, Se, and thiobarbituric acid reactive substances (TBARS), along with serum activity levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx), were measured. Although serum F levels were higher both before ($p<0.001$) and after ($p<0.002$) HD than in the conservatively treated group, HD resulted in a statistically significant ($p<0.005$) decrease of the F level. In both patient groups, a positive correlation was found between the levels of serum F and TBARS. In patients undergoing HD, a negative correlation was observed between the serum F level before HD and SOD activity ($p<0.01$) on the one hand and copper levels ($p<0.0004$) on the other. In the conservatively treated patients not undergoing HD, the GPx activity level in the serum was positively correlated with the F level. Thus an oxidation promoting action of F in patients with CRF was confirmed.

Keywords: Chronic Renal Failure; Fluoride stress; Glutathione peroxidase; Haemodialysis; Plasma copper; Plasma selenium; Plasma zinc; Superoxide dismutase; Thiobarbituric Acid Reactive Substances.

INTRODUCTION

Elimination of ionic fluoride (F) is impaired in chronic renal diseases.[1-4] As a result, plasma F increases,[1,2,4,5] and F accumulates in the body. In treatment of chronic renal failure (CRF), the common method of restoring the correct water and electrolyte balance in the body and eliminating toxins is haemodialysis (HD). The procedure is based on the phenomenon of penetration of some low and medium molecular weight substances through a semi-permeable membrane and a balancing of concentrations of these substances on both sides of the membrane. In HD, F is not fully removed from the plasma,[3,6-8] and its clearance is lower than that of other substances.[9,10] The ionic radius of F (1.3×10^{-10} m) is smaller than the pore diameter of the dialysis membrane ($5–50\times10^{-10}$ m), yet the fact that F remains in the body despite effective haemodialysis indicates F is not present in the plasma in a simple ionized form but is probably bound to proteins[10] or to other substances, including substances contained in blood cells.[11]

Within the first hour of HD, serum F levels become stable, commonly attaining normal values. A logarithmic correlation between F and the duration of haemodialysis has been reported.[12]

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Increasingly effective methods of purification of the water used in dialysis therapy for CRF have largely reduced the possibility of introducing F into the patient bodies in the course of haemodialysis.[13] Until recently, F was associated in dialysis therapy with chronic[14-16] or acute intoxications.[8,13,17,18] However, the discovery of oxidation-promoting properties of F[19] emphasizes the need to alleviate oxidative stress in patients with CRF, whether treated conservatively or with HD. In these patients, activated neutrophils and monocytes are most commonly the source of reactive oxygen species (ROS).

Thus the positive effect of HD that purifies the body from uremic toxins is also accompanied by a number of side effects. Plasma proteins, primarily albumins, and red blood cells especially are exposed to the destructive action of highly reactive oxygen compounds formed in the course of HD. Hypoalbuminaemia is associated with increased mortality of dialysed patients[20] and with increased peroxidation of lipids of erythrocyte cell membranes.[21]

Superoxide anion-radical formed by stimulated neutrophils is converted into hydrogen peroxide by the action of superoxide dismutase (SOD). The resulting hydrogen peroxide is decomposed by the action of glutathione peroxidase (GPx).

The basic marker of oxidative stress is the level of TBARS – thiobarbituric acid reactive substances (most often dialdehydes). Their formation results from degradation by free radicals of polyunsaturated fatty acids present in lipids.[23] Although much more elevated in patients undergoing HD,[21,22] TBARS plasma levels are less elevated in conservatively-treated patients with renal failure.[22,23]

The available literature lacks reports that would associate the pro-oxidative action of F with the creation of oxidative stress in patients with CRF treated either conservatively or by repeated HD.

MATERIALS AND METHODS

The investigation was conducted on group of 33 patients (16 women and 17 men, age 58.45 ± 14.93 years) subjected to chronic HD with Fresenius polysulphonic dialysers. The patients were dialysed three times a week, usually with a 4-hr HD session. The aqueous dialysis solution (after the concentrate was diluted) had the following composition: 138 mmol/L Na^+, 0, 2.0 or 3.0 mmol/L K^+, 1.75 mmol/L Ca^{2+}, 0.5 mmol/L Mg^{2+}, 107.5 mmol/L Cl^-, 0 or 5.5 mmol/L glucose, and 32 mmol/L HCO_3^-. The water used for haemodialysis was obtained by reverse osmosis. F levels in this water used for HD haemodialysis and in the preceding concentrate were below the limit of measurement with the F ion-selective electrode.

Renal failure was caused by glomerulonephritis in 12 cases, pyelonephritis in 14 cases and by other causes in 7 cases. The control group consisted of 19 persons (6 women, 13 men, age 55.69 ± 13.46 years) with conservatively-treated CRF, including 7 with glomerulonephritis, 6 with pyelonephritis, and 6 with other pathologies. These control patients, not yet eligible for HD, were seen monthly in the outpatient clinic and were treated conservatively with angiotensin-converting enzyme inhibitors and diuretics.

None of the patients in both groups had blood transfused within a few weeks preceding the study. The patients did not have any malignancies or active

inflammatory conditions. None of the patients were smokers or used microelement supplementation. The study was approved by the Bioethics Committee of the Pomeranian Medical University. All patients gave their consent to participate in the study.

Blood was collected from an antecubital vein in the morning before breakfast from the conservatively treated CRF patients. In the patients undergoing HD, blood was collected immediately before the beginning and immediately after the termination of HD from the arteriovenous fistula.

Blood samples were collected with heparin (50 IU/mL) present as an anticoagulant. F in the plasma was measured potentiometrically with an Orion F ion-selective electrode after prior addition of TISAB (Total Ionic Strength Adjustment Buffer) to the plasma. The sensitivity of F plasma level measurement by this method is 10^{-6} mol/L (0.019 mg/L). The concentration of thiobarbituric acid reactive substances (TBARS) in the plasma was measured by spectrofluorometry.[24] The level of glutathione peroxidase (GPx) activity in the plasma was measured by an enzymatic method.[25] Superoxide dismutase (SOD) in erythrocytes was measured by spectrophotometry.[26] Selenium (Se) in the plasma was measured by spectrofluorometry.[27] Copper (Cu) and zinc (Zn) plasma levels were measured by atomic absorption spectrometry with the use of a Philips PU 9100X absorptiometer.

All results are presented as the mean value ± standard deviation (SD). After it was found with the use of the Shapiro-Wilk test that the distributions of results obtained were not normal, the following non-parametric tests were used for statistical analysis: Wilcoxon matched pairs test for examining the differences in parameters before and after dialysis, Mann-Whitney U rank analysis test to determine the dependence between patient groups with chronic renal failure treated conservatively and those treated with HD, and Spearman's test to determine the correlation between the parameters measured.

RESULTS AND DISCUSSION

Biochemical test results – TBARS, SOD, GPx, their cofactor levels, and other data characterizing the study groups – are presented in the Table. F levels and statistically significant relations are presented in Figure 1.

Plasma F levels in patients with CRF treated conservatively did not differ from those measured by the F ion-selective electrode in 63 healthy persons residing in the same geographical region.[28] Patients with CRF start dialysis therapy when creatinine clearance (glomerular filtration rate, GFR) is less than 10 mL/min/1.73 m^2. Until the GFR is above this value, they are treated conservatively in Outpatient Clinic. In the plasma of dialysed patients, the F level was significantly higher than in the group treated conservatively. This phenomenon was observed both before and after HD. However, HD caused a statistically significant lowering of the plasma F level ($p<0.005$), although after HD plasma F levels remained higher than in patients treated conservatively. This finding confirms reports that only ca. 60% of plasma F is removed by HD. The rest is apparently bound with species that do not undergo filtration.[29] An increase in F level, including the ionised fraction, is encountered not only in patients treated by HD but also in those

treated by peritoneal dialysis. F clearance in the course of peritoneal dialysis is higher and may attain even up to 90%.[30] Plasma F levels in patients treated by HD as well as those treated conservatively did not correlate with either the age or gender of the patients. Moreover, the time from the introduction of renal replacement treatment (first HD) and the amount of dialyser use did not affect plasma F levels. We did not confirm the report of a statistically significant increase in plasma F level with the passage of time from commencement of the first dialysis.[12]

Table. Selenium, copper, zinc, and TBARS concentration in plasma and SOD and GPx activity in erythrocytes. Normal values of selenium, copper, zinc, and TBARS concentration in plasma and SOD and GPx activity in erythrocytes.[45]

Parameter	CRF group before HD	CRF group after HD	CRF group without HD	Normal values (min – max)
TBARS (µmol/L)	1.43 ± 0.3	1.50 ± 0.58*	1.05 ± 0.21*	0.60 – 1.05
SOD (U/g Hb)	1.73 ± 0.16	1.77 ± 0.14†	1.69 ± 0.096†	1.65 – 1.97
GPx (U/g Hb)	7.79 ± 1.70	8.44 ± 2.23*	9.46 ± 1.23*	4.16 – 10.5
Cu (µmol/L)	14.31 ± 8.81	14.62 ± 8.33	17.79 ± 3.46	13.0 – 22.0
Zn (µmol/L)	13.06 ± 3.06	14.91 ± 2.90	8.39 ± 3.55*	2.0 – 23.0
Se (µmol/L)	0.58 ± 0.19	0.61 ± 0.20†	0.55 ± 0.18	0.37 – 0.79
Urea nitrogen (mmol/L)	43.2 ± 8.04	17.1 ± 6.1†	12.4 ± 10.7†	7.1 – 16.4
Creatinine (µmol/L)	840.2 ± 256.5	450.2 ± 147.7†	258.3 ± 124.7†	61.9 – 115
No. times dialysator used	6.2 ± 4.5			
Time since first HD (months)	3.64 ± 0.47			
Age of patient (years)	58.45 ± 14.93		55.69 ± 13.46	

Values are means ± SD. Statistically significant changes after HD vs. before HD are marked as: * = p<0.05, † = p<0.01. Statistically significant changes between groups after HD and without HD are similarly marked.

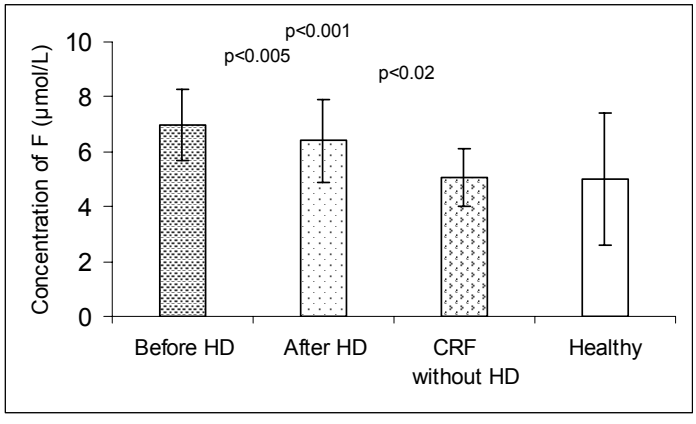

Figure 1. Plasma F concentration of CRF patients treated with or without HD and healthy, non-CRF patients.[28] Values are shown as means ± SD.

Serum TBARS levels (Table 1) were significantly higher in HD patients than in patients treated conservatively, in accord with findings by other authors.[21-23] A positive correlation was found between F and TBARS levels in patients treated

conservatively as well as in those treated by HD (Figure 2). F correlates with the TBARS level not only before HD but also, despite lowering of its level, after HD. An increase in the TBARS level in animals drinking water containing F has been confirmed in numerous studies. Water containing up to 100 ppm F causes an increase in peroxidation of lung tissue[31] and renal tissue[32] in rats. Peroxidation of erythrocyte cell membrane was observed in rats drinking water containing 100 ppm F but not at 30 ppm.[33]

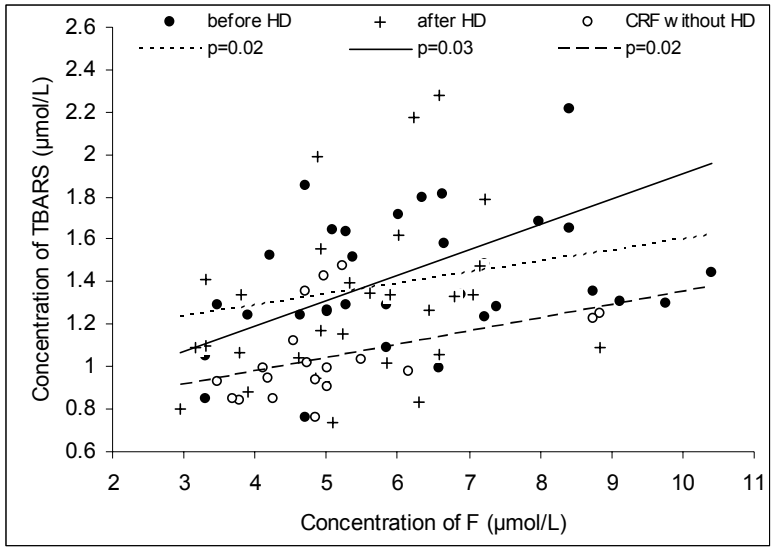

Figure 2. Correlation between F and TBARS concentrations in plasma.

Our results show that serum SOD levels increased in a statistically significant fashion in the course of HD and was higher than in the patient group treated conservatively. Toborek et al.[34] and Canestrari et al.,[35] measuring serum SOD activity in HD patients, found an increased SOD before HD, which is lowered in the course of HD. The consequence of this is increased lipid peroxidation. Different results were obtained by Mimic-Oka et al.,[36] which showed increased serum SOD activity in CRF patients that correlated with advancement stage of the disease, but in HD patients they found marked lowering of SOD level.[36]

The dependence found between serum F levels and SOD activity is interesting (Figure 3). A statistically significant inverse correlation was found in HD patients but not in patients treated conservatively. Lowered activity of erythrocytic SOD is observed in animals drinking water containing F at high (100 ppm) but not at low concentrations (30 ppm), which had increased levels of this enzyme.[33] Reduced SOD levels were found by Shivarajashankara et al.[41] but not by Reddy et al.,[38] who did not detect any differences in patients with fluorosis and in rabbits on fluoride-high diet comparison with normal diet.

Superoxide dismutase is a metalloprotein, the activity of which depends on the presence of Cu and Zn. It is usually assumed that renal diseases are associated with reduced Cu levels.[23,39] In our work, no statistically significant differences were found in serum Cu levels before and after HD as compared with the group treated conservatively. Zn levels of the plasma of dialysed patients were

significantly higher than in the group treated conservatively. The levels of both microelements did not change in the course of HD. No statistically significant correlations were found between the levels of Zn (structural element of SOD) and F. In contrast, Cu, an element that has a catalytic role in SOD, behaves similarly in the enzyme under the influence of F. Before and after HD, we found a strongly negative correlation between F and Cu levels (p=0.0004 before HD and p=0.0001 after HD). This correlation, however, was not seen in patients treated conservatively. Fluctuations in levels of these elements were evident in the bodies of rats on a fluoride diet. In the liver, the levels of Cu and Zn were lowered, in kidneys the Zn level was elevated, and in bones the Cu concentration decreased.[40] F probably binds by ionic forces with Cu in the active center of SOD.[41]

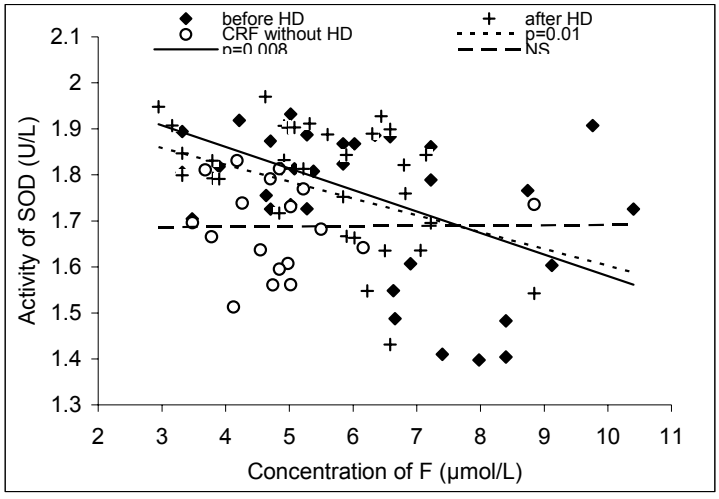

Figure 3. Correlation between plasma F concentration and activity of SOD in erythrocytes.

The serum glutathione peroxidase level in our HD patients did not depend on the F level, but such dependence was positive and statistically significant in the group treated conservatively (Figure 4).

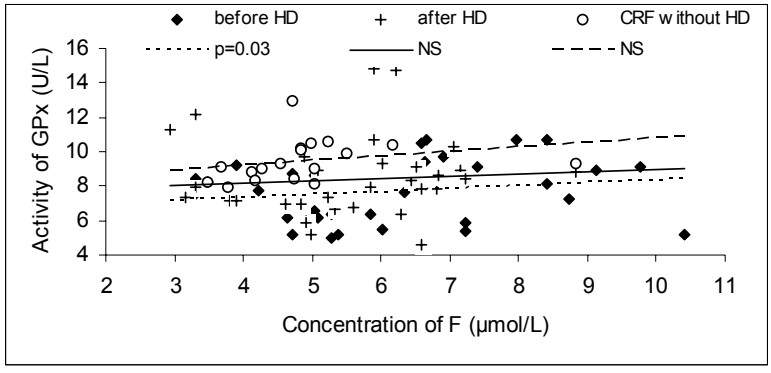

Figure 4. Correlation between plasma F concentration and activity of GPx in erythrocytes.

In animals drinking water containing F the GPx level increased irrespectively of the F level.[32,39] Different results were obtained by Krechniak et al.,[42] who found a negative correlation between F level and GPx activity, and Reddy et al.,[38] who did not observe any changes in GSH and GPx activities in both persons with fluorosis and in rabbits on a diet high in F.

The cofactor of glutathione peroxidase is selenium. In dialyzed patients a deficiency of Se is often noted, which may result from its loss through the dialysis membrane or its dietary deficiency.[39] F levels did not correlate in a statistically significant fashion with the Se plasma levels.

In conclusion, our results indicate a direct correlation between serum F levels and the intensity of oxidative stress in patients with CRF and at the same time with stimulation of adaptation mechanisms of the body in response to increased levels of this ion.

Although water purification by micro-osmosis protects against the accumulation of trace elements in the body,[43] the role of microelements in patients undergoing HD is not completely elucidated. Appropriate preparation of water is of fundamental importance for the correct performance of haemodialysis.[44]

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