ADVANCED OXIDATION PROTEIN PRODUCTS (AOPP) LEVELS AND KIDNEY FUNCTION IN FLUOROTIC SHEEP

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ABSTRACT: The ingestion of excessive amounts of the fluoride ion (F) may cause chronic fluorosis with abnormalities in many organs and systems including, in addition to dental and skeletal fluorosis, impaired renal function. The aim of the present study was to investigate renal function and the levels of advanced oxidation protein products (AOPP) as oxidative stress markers in chronically fluorotic sheep with kidney disease. Fifteen healthy and 30 fluorotic sheep were studied. The plasma F levels were determined with a F-selective electrode. The plasma AOPP and the serum total protein levels were measured spectrophotometrically. The serum BUN, creatinine, potassium, sodium, and chloride levels were determined by autoanalyzer. Compared to the control group, significant increases were present in the fluorotic sheep in the levels of F (p ≤ 0.001), AOPP (p ≤ 0.001), BUN (p ≤ 0.001), creatinine (p ≤ 0.05), and total protein (p ≤ 0.05). A significant decrease was present in the fluorotic sheep in the level of potassium (p ≤ 0.01) while no significant changes were present in the sodium and chloride concentrations. The levels of AOPP in chronically fluorotic sheep, reported here for the first time in veterinary science, and the biochemical abnormalities can be considered in the evaluation of the effects of chronic fluorosis on kidney function.

Key words: Advanced oxidation protein products; Biochemical abnormalities; Fluoride; Kidney; Sheep.

INTRODUCTION

Fluoride intake in human occurs through fluoridated water, pesticides, dental restorations, post-harvest fumigants, some foods and ambient air. Endemic fluorosis may occur due to a high fluoride ion (F) concentration in the drinking water or food, while industrial fluorosis is mainly related to airborne fluoride in industrial working environments.1,2 A daily intake of F of 0.5 to 1.7 mg F/kg body weight (bw), in the form of sodium fluoride, produces dental lesions in growing animals.3 The skeleton, teeth, brain, liver, kidneys, and spinal cord are the main targets of F.3,4,5 High F levels interfere with mineral metabolism and cause an abnormal growth of bone that may be structurally weak. F can also damage the parathyroid glands, leading to hyperparathyroidism, and decrease bone flexibility making the bone more susceptible to fractures.5 Mechanical neurological complications may occur in endemic skeletal fluorosis, namely radiculopathy, myelopathy, or both.6 F was noted in 2006 to cause neurotoxicity in laboratory animals and was described as an emerging neurotoxic substance.7 Fluoride may be a developmental neurotoxicant that affects brain development at exposures much below those that can cause toxicity in adults. Fluoride neurotoxicity may target hippocampal neurons.7 In 2014 F was described as a newly recognized

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developmental neurotoxicant with an average IQ decrement of 7 points being found in a meta-analysis of 27 cross-sectional studies of children exposed to F in drinking water, mainly from the People’s Republic of China. Dental fluorosis has been seen to be marker for F-induced cognitive impairment.8,9 Urine is the main route for the excretion of F. In consequence, chronic fluorosis results in impaired renal function, which in turn leads to an increased plasma F concentration and accumulation in several organs.11 Acute exposure to high F concentrations also leads to kidney tissue damage.12

At the molecular level, F promotes the formation of free radicals, inhibits the activity of antioxidant enzymes, and increases lipid peroxidation. Water containing up to 100 mg F/L (ppm) results in increased peroxidation in lung and kidney tissues and on the cell membrane of erythrocytes. A direct correlation has been found between the serum F and the intensity of oxidative stress in chronic renal failure (CRF).11 These results suggest that F toxicity is related to the production of free radicals and an alteration of the antioxidant defense system.

In a similar manner to enzymes and metabolites, AOPP also reflect oxidative stress. AOPP occur by the oxidation of plasma proteins and accumulate with renal and coronary diseases.13 A significant correlation has been found between the level of AOPP and the creatinine clearance indicating that AOPP are a good marker of the progression of chronic renal failure.14

In the Eastern part of Turkey, fluorosis is a serious health problem for both humans and animals who consume F from water from near the volcanic areas of Mt Ararat and Mt Tendurek. To date, there have been few studies of the effect of F on renal function in sheep. Therefore, the main objective of this research was to investigate renal function and AOPP levels as oxidative stress markers in chronically fluorotic sheep with kidney disease.

MATERIALS AND METHODS

Thirty Morkaraman sheep, aged 3–5 yr, with chronic fluorosis, raised in the Eastern Turkey cities of Van and Agri and their surrounding areas, where tooth and bone deformities in sheep are widely observed, were used in the study as these ages are sufficient for the development of fluorosis. Chronic fluorosis was diagnosed in the sheep by blood measurements of F and clinical examination with verification of the presence of lameness, swollen joints, tooth discoloration, and dental erosions. The sheep were fed in extensive pasture.

Fifteen healthy Morkaraman sheep, aged 3–5 yr, obtained from the Research Farm of the Faculty of Veterinary Medicine, Yuzuncu Yil University in Van, were used as controls. They were fed in extensive pasture far from the fluorotic area. Their drinking water was not contaminated with F and they were all clinically healthy.

The absence of parasitic diseases from all the sheep was confirmed by faecal parasitological examination.

Blood samples were collected in plain and heparinized tubes from the vena jugularis. The blood was centrifuged at 3000 rpm for 15 min to separate the serum and plasma.
A F-sensitive electrode (Fluoride electrode Orion, Thermo Scientific, USA) was used to measure the concentration of plasma F following a standard analytical technique. The plasma AOPP levels were determined spectrophotometrically (Perkin Elmer, USA) with absorbance at 340 nm and were expressed in chloramine units (µmol/L). Two hundred µL of plasma diluted 1:5 in PBS, or chloramine-T standard solutions (0 to 100 µmol/L), were prepared and 20 µL of acetic acid added. Ten µL of 1.16 M potassium iodide were then added, followed by 20 µL of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against a blank containing 200 µL of PBS, 10 µL of KI, and 20 µL of acetic acid. The chloramine-T absorbance at 340 nm was linear within the range of 0 to 100 µmol/L. The AOPP concentrations were expressed in µmol/L of chloramine-T equivalents. The serum total protein levels were also measured by spectrophotometric methods. The serum levels of creatinine, Na⁺, K⁺, Cl⁻, and BUN were determined by means of an autoanalyzer (Roche, Modular P800, USA).

As the unequal variance t-test should always be used in preference to the Student’s t-test for the measurement of the central tendency of 2 populations based on samples of unrelated data, the data were evaluated statistically by the unpaired t-test using the Statistical Packages for the Social Sciences (SPSS 16.0) for Windows.

RESULTS

The results of the biochemical analyses are presented in Table 1.

Table 1. Biochemical parameters in healthy (control) and fluorotic sheep.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy sheep (control) (n=15, X±SEM*)</th>
<th>Fluorotic sheep (n=30, X±SEM*)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride (µmol/L)</td>
<td>6.84±0.37</td>
<td>18.95±0.53</td>
<td>≤0.001</td>
</tr>
<tr>
<td>AOPP (µmol/L)†</td>
<td>99.84±6.85</td>
<td>147.58±7.16</td>
<td>≤0.001</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>8.41±0.58</td>
<td>18.33±0.90</td>
<td>≤0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.78±0.03</td>
<td>0.90±0.03</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.30±0.39</td>
<td>6.44±0.27</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Na⁺ (mmol/L)</td>
<td>137.47±1.91</td>
<td>138.83±1.12</td>
<td>NS</td>
</tr>
<tr>
<td>K⁺ (mmol/L)</td>
<td>5.99±0.21</td>
<td>5.06±0.17</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Cl⁻ (mmol/L)</td>
<td>108.47±1.65</td>
<td>107.73±0.80</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are mean±standard error of mean (SEM); †AOPP: advanced oxidation protein products.

Compared to the control group, significant increases were present in the fluorotic sheep in the levels of F (p≤0.001), AOPP (p≤0.001), BUN (p≤0.001), creatinine (p≤0.05), and total protein (p≤0.05). A significant decrease was present
in the fluorotic sheep in the level of potassium (p≤0.01) while no significant changes were present in the sodium and chloride concentrations.

**DISCUSSION**

Because many places in East Anatolia in Turkey are covered with volcanic ash, some trace elements are absent while others are present in abundance, such as with F which occurs in the drinking water, soil, and flora of the region. The natural water resources are rich in F and for many years endemic fluorosis has been known to occur in this region. The level of F in drinking water in the region ranges from 5.7 to 15.2 mg F/L (ppm). Dobaradaran et al. studied the relationship between the groundwater F concentration and dental caries in children in the Dashtestan area of the Bushehr Province in Iran and found that the village drinking water F level ranged from 0.99 to 2.50 mg F/L. The F concentrations in 17 brands of bottled drinking water in Iran were also determined and found to have a mean of 0.3 mg F/L and a range of 0.00–0.59 mg F/L. It is well known that an excessive intake of F in fluoridated water or in the diet causes health problems. The plasma level of F in control sheep and goats was reported by Bennis et al. to be approximately 0.10 mg F/L (5.26 µmol/L). Although Bennis et al. gave the F level in fluoride intoxication in sheep as up to 1.3 mg F/L (68.42 µmol F/L) during acute F intoxication, the paper they give as a reference by Kessabi et al. shows, in Figure 1, the steady state serum F levels in experimental fluorosis in sheep, given 0.10 and 0.25 mmol F/kg bw for 2 to 33 months, to be approximately 85 µmol F/L (1.6 mg F/L) and 45 µmol F/L (0.86 mg F/L), respectively. Oto and Turel reported plasma F levels in the 23.16–48.95 µmol F/L range in sheep in a high-F region in Eastern Turkey. In the present study, the plasma concentrations of F in the healthy and fluorotic sheep were 6.84±0.37 and 18.95±0.53 µmol F/L, respectively (p≤0.001).

Excess reactive oxygen species (ROS) production in cells after exposure to a toxin can overwhelm the antioxidant defense mechanisms and damage cellular ingredients such as lipids, proteins, and DNA. This in turn can impair cellular structure and function. The extent of lipid peroxidation and protein carbonylation are additional indicators of tissue oxidative injury. The increase in ROS induced by NaF suggests that F causes a higher degree of oxidative stress and extensive cellular damage in tissues. Das et al. found that F induced a significant reduction in the antioxidant power of hepatocytes as indicated by the lower ferric reducing/antioxidant power value compared to that in the normal hepatocytes. In addition, they assessed the effect of F on lipid peroxidation and protein carbonyl content.

Plasma proteins are the main targets of oxidants and, as a result, AOPP are a risk factor in kidney disease and important markers of oxidative stress in uremia, where AOPP act as a mediator of oxidative stress. Witko-Sarsat et al. suggested that AOPP could contribute to the monocyte-mediated inflammatory disorders associated with uremia. In addition, they proposed that the level of AOPP could be a reliable marker for estimating the degree of oxidant-mediated protein damage in uremic patients and for predicting the potential efficacy of therapeutic strategies aimed at reducing such an oxidative stress. In our work we determined that the
AOPP concentration was significantly increased (p≤0.001) due to the oxidation of protein during fluorosis.

The kidneys are the primary organs concerned with the excretion and retention of F and thus are generally involved in chronic F intoxication. F is concentrated to much higher levels in the kidney tubules than it is present in the plasma. With higher NaF doses, the cytoarchitecture of the kidneys exhibit increasing amounts of cloudy swelling, degeneration of the tubular epithelia, tissue necrosis, extensive vacuolization in the renal tubules, hypertrophy and atrophy of the glomeruli, exudation, interstitial oedema, and interstitial nephritis. In addition, Kono et al. reported impaired renal function in F-exposed workers.

The serum levels of creatinine and BUN are used commonly as markers of kidney function. The serum creatinine reflects the ability of the kidney to remove creatinine from the blood and to concentrate it in the urine. Renal dysfunction diminishes the ability to filter creatinine and the serum creatinine rises. Diseased or damaged kidneys also cause an elevated BUN because the kidneys are less able to clear urea from the bloodstream. Bouaziz et al. reported that the plasma level of creatinine was higher in adult mice exposed to NaF, compared to the controls (p≤0.001). Akdogan et al. found a significant increase in the level of BUN and creatinine in fluorotic rabbits, which was greater with higher doses of F. In the present study, the BUN (p≤0.001) and creatinine (p≤0.05) levels were increased in the fluorotic sheep.

F inhibits protein synthesis mainly due to an impairment of peptide chain initiation and by interfering with peptide chains on ribosomes. Michael et al. reported that F may disturb protein synthesis and elevate the activities of alanine transaminase (ALT) and aspartate transaminase (AST). The enhanced levels of these serum transaminases, which are markers for liver function, indicates structural and functional changes in the liver due to the F intake. Shivashankara et al. found a slight but significant decrease in serum total protein and albumin levels in chronic F toxicity in children. However, in the present study the total protein levels were significantly increased (p≤0.05) in the fluorotic sheep. A high F concentration may be toxic for the liver resulting in structural and functional changes. All the serum proteins except immunoglobulin are synthesized in the liver cells. In a study of male mice, severe liver damage induced by carbon tetrachloride (CCl4) resulted in a decrease in protein catabolism and an elongation of the serum protein half life in order to compensate for the decrease in the protein synthesis.

In the present study, there were no changes in the sodium or chloride concentrations while the potassium level decreased significantly, compared to controls (p≤0.01). In contrast, Michael et al. observed an elevation in the serum potassium level in fluorotic individuals. Suketa and Terui suggested that adrenal function plays an important role in fluorosis by maintaining a strong homeostasis in the ionic mobilization of sodium and potassium. However, Das and Susheela reported low corticosteroid levels in fluorotic humans, suggesting adrenal hypofunction.
CONCLUSIONS

In conclusion, our results confirm that chronic fluorosis affects kidney function. As well as F-induced nephrotoxicity causing elevated serum levels of BUN and creatinine, it also raises the plasma AOPP. The present study is the first report of the use of AOPP, in addition to the other biochemical changes, for assessing renal damage in chronically fluorotic sheep.

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REFERENCES