EVALUATION OF SERUM LIPOPROTEIN AND TISSUE ANTIOXIDANT LEVELS IN SHEEP WITH FLUOROSIS

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SUMMARY: The aim of this study was to evaluate serum lipoprotein and tissue antioxidant levels of sheep with and without fluorosis living in a volcanic area of Turkey. Fifteen Akkaraman sheep with fluorosis in the Ağrı region north of Lake Van and 10 Akkaraman sheep without fluorosis in the Van region just south of Lake Van in the eastern part of Turkey were investigated. In the kidney tissues, the MDA levels and SOD activities in the fluorosed sheep showed nonsignificant increases, but the GSH level and GPx activities significantly decreased. In the liver tissues of the fluorosed sheep, a significant increase in the MDA level and GPx activity was observed, but the GSH level showed no change, and the SOD activity exhibited a small decrease. By contrast, in the muscle tissues, the MDA level decreased and the SOD activity increased significantly, whereas the GPx activity increased but not significantly, and the GSH levels decreased. Finally, the serum lipoprotein levels of the fluorosed and nonfluorosed sheep were not significantly different. In conclusion, different degrees in the pro-oxidant/antioxidant status of soft tissues such as kidney, liver, and muscle were affected by F intoxication, but no differences were found in the serum lipoprotein levels.

Keywords: Akkaraman sheep; Endemic fluorosis; Kidney; Liver; MDA; Muscle; Serum lipoproteins, Sheep in Turkey.

INTRODUCTION

Chronic fluorosis caused by long-term exposure to high levels of fluoride (F) is often observed in areas of volcanic activity and in regions where industrial waste contaminates water and soils. 1 In the far eastern part of Turkey, chronic fluorosis is an endemic health problem, as in the area of Ağrı north of Lake Van where high F levels in the groundwater are the result of volcanic eruptions that occurred many years ago. 2-5

Various studies 6-9 have reported that fluorosis causes decreased activity of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) in different tissues, along with changes in the level of reduced glutathione (GSH) and an increase in the level of malondialdehyde (MDA), which is an indicator of lipid peroxidative damage and also changes in membrane lipid and lipid composition. Moreover, F intoxication has been found to disturb normal lipid metabolism to varying degrees. 10-12 However, plasma lipid and lipoprotein levels are also affected by intrinsic factors such as age, body weight, genetic heritage, and gender as well as by environmental influences such as diet and physical activity. 13

The aim of this study was to evaluate serum lipoprotein levels and kidney, liver, and muscle antioxidant levels of sheep with fluorosis in the endemic volcanic fluoride region of Ağrı located north of Lake Van in eastern Turkey.
MATERIALS AND METHODS

Animal material: Ayranç village of Doğubeyazıt, Ağrı, Turkey, was chosen as the study area in which fluorosis is endemic. Fifteen two-year-old or older Akkaraman sheep were selected for the experimental material. To determine the presence of fluorosis and history of fluorosis, signs of fluorosis by clinical examination of teeth and joints were evaluated. Urine F levels of the animals were also determined. For controls, ten Akkaraman sheep of the same age without fluorosis as determined by clinical and laboratory examination were selected from the city area of Van located south of Ağrı.

Determination of fluoride: Blood and urine samples were collected by suction from the sheep into specimen tubes. Serum of the samples was separated by centrifuging at 3000 rpm for 10 min. Urine samples were collected in polyethylene tubes, and F levels were immediately determined with an ion selective electrode (WTW PH / ION 738®).14

Biochemical analysis: Kidney, liver, and muscle samples were taken after slaughtering the animals and saved for analysis at −80°C. These tissues were extracted for determination of MDA, GSH, SOD, and GPx by the method reported by Kahraman.15 Determination of MDA levels was based on the amount of colored product resulting from reaction between thiobarbituric acid (TBA) and MDA as described by Ohkawa et al.16 Tissue GSH levels17 were estimated with a spectrophotometer by the color created by free-sulfhydryl groups reacting with the Elman reagent. SOD and GPx activities were determined with commercial kits (Randox-Ransod enzyme kit) with a spectrophotometer.18,19

Electrophoresis analysis: Serum lipoproteins in the serum samples were fractionated by the cellulose acetate electrophoresis method. The optical density of the lipoprotein fractions was measured at 525 nm by the Helena electrophoresis procedure.20

Statistical analysis: The Student t test was used to determine statistical differences between the control and fluorosis groups. Differences with p<0.05 were accepted as significant.

RESULTS

As can be calculated from Table 1, the mean urinary F level of the sheep with fluorosis was 4.5 times higher than that of the control sheep without fluorosis, a difference that is highly significant.

Table 1. Urinary F levels of sheep with and without fluorosis (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Fluorosis group (n=15)</th>
<th>Control group (n=10)</th>
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</thead>
<tbody>
<tr>
<td>Urinary F (ppm)</td>
<td>6.74±0.49*</td>
<td>1.50±0.30</td>
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</table>

*p<0.001 in comparison to control.
Although MDA levels in the kidney tissues of sheep with fluorosis were not significantly different from the control group, they were numerically greater. The GSH level of the fluorosed sheep was significantly lower than in the control group. GPx and SOD activities were higher, with the former being significantly higher (Table 2).

### Table 2. MDA and GSH levels, and SOD and GPx activities in kidney tissues of sheep with and without fluorosis (mean±SD)

<table>
<thead>
<tr>
<th>Kidney tissue</th>
<th>Fluorosis group (n=15)</th>
<th>Control group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>298.27±12.34</td>
<td>286.15±12.61</td>
</tr>
<tr>
<td>GSH (µmol/g tissue)</td>
<td>0.212±0.024*</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>137.45±17.13</td>
<td>101.59±5.69</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>2.22±0.29*</td>
<td>0.95±0.08</td>
</tr>
</tbody>
</table>

*p<0.05 in comparison to control.

In the liver, the MDA level significantly increased in sheep with fluorosis, but the GSH level increased only slightly. Liver tissue SOD activity decreased nonsignificantly, whereas GPx activity increased significantly in sheep with fluorosis (Table 3).

### Table 3. MDA and GSH levels, and SOD and GPx activities in liver tissues of sheep with and without fluorosis (mean±SD)

<table>
<thead>
<tr>
<th>Liver tissue</th>
<th>Fluorosis group (n=15)</th>
<th>Control group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>404.85±11.53*</td>
<td>352.09±11.32</td>
</tr>
<tr>
<td>GSH (µmol/g tissue)</td>
<td>0.404±0.02</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>479.72±30.80</td>
<td>497.01±14.34</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>3.78±0.48*</td>
<td>2.16±0.22</td>
</tr>
</tbody>
</table>

*p<0.05 in comparison to control.

In contrast to the kidney, the MDA level in the muscle tissue of the fluorosed sheep showed a significant decrease. The SOD activity, however, was significantly higher compared with the control group, while the GSH level was lower, and the GPx activity was higher. (Table 4).

### Table 4. MDA and GSH levels, and SOD and GPx activities in muscle tissues of sheep with and without fluorosis (mean±SD)

<table>
<thead>
<tr>
<th>Muscle tissue</th>
<th>Fluorosis group (n=15)</th>
<th>Control group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>153.38±6.51*</td>
<td>165.89±3.97</td>
</tr>
<tr>
<td>GSH (µmol/g tissue)</td>
<td>0.042±0.005</td>
<td>0.174±0.072</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>194.25±27.59*</td>
<td>85.65±7.07</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>1.90±0.27</td>
<td>1.34±0.17</td>
</tr>
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</table>

*p<0.05 in comparison to control.
As seen in Table 5, the concentrations of the serum lipoprotein fractions of sheep with and without fluorosis were virtually unchanged without any significant differences.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Fluorosis group (n=15)</th>
<th>Control group (n=10)</th>
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</thead>
<tbody>
<tr>
<td>β-lipoprotein (LDL) %</td>
<td>31.09±2.02</td>
<td>29.53±2.53</td>
</tr>
<tr>
<td>Pre-β-lipoprotein (VLDL) %</td>
<td>38.81±3.40</td>
<td>40.90±3.94</td>
</tr>
<tr>
<td>α-Lipoprotein (HDL) %</td>
<td>29.21±3.79</td>
<td>27.84±1.47</td>
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**DISCUSSION**

Normal urinary F levels of sheep are reported to be 1.00–1.40 ppm. There is also a positive correlation between the amount of F ingested by animals and humans and the level of F in their urine, which is usually less than 5 ppm F. Şendil and Bayşu have reported that urinary F levels of sheep in Eastern Anatolia, where endemic fluorosis is common, ranged from 3.80 to 30.6 ppm. However, the average urinary F the level of sheep raised in that region is reported by Ergun et al. as 8.1 ppm. In the present study, the mean urinary F level in the sheep with fluorosis was 6.74±0.49 ppm, which is in agreement with the results for sheep in natural endemic F areas reported by Altıntaş et al. Urinary F levels (1.50±0.30 ppm) in the control group of this study are also in agreement with values reported in the literature.

As a highly toxic pollutant, F affects the metabolism of many tissues and organs besides bones and teeth. Liver and kidney are also important target organs for F in both acute and chronic fluorosis. Many current experimental and epidemiological studies shown that chronic fluorosis can cause metabolic, functional, and structural damage to the kidney and liver, and to endocrine glands (hypophysis, thyroid, and parathyroid), muscle, testis, and other soft tissues including neurons. As a center of metabolic activity, the liver plays a key role in F metabolism. The kidney is an organ of F excretion that is more subject to failure with exposure to increased levels of ingested F, leading to increased serum F levels in humans and experimental animals. Kidneys, along with calcified tissues, act as a homeostasis control for F, and, in endemic fluorosis areas, there is a relationship between kidney function and environmental F intoxication. It is well established that impairment of kidney function can occur in both chronic and acute fluorosis.

There is also evidence that oxidative stress and changes in cell membrane lipid composition play a role in the pathogenesis of kidney tissue damage during acute and chronic F exposure. Increased MDA (TBARS) levels in kidneys have been found as the result of F treatment, indicating lipid peroxidative damage in kidney tissue. With elevated levels of F intake, the liver undergoes functional and structural changes. In addition to changes in membrane-lipid composition caused by F, decreases in antioxidant enzyme activities of SOD and GPx, and
increases in MDA level as an indicator of lipid peroxidative damage in different tissues have been reported.6-9

In the present study, the antioxidant/pro-oxidant status of fluorotic sheep tissues was affected differently. Liver MDA levels were significantly higher in sheep with fluorosis than in the control group without fluorosis, but the GSH levels were similar. The liver SOD activity decreased, and the GPx activity increased significantly in the sheep with fluorosis. On the other hand, muscle MDA levels were significantly lower, and SOD activities were significantly higher in sheep with fluorosis compared with the sheep without fluorosis. However, the muscle GSH level decreased, and the GPx activity increased.

In contrast to these findings, Rice et al.29 reported that GPx activity decreased in the liver of rats due to excessive accumulation of free-radicals in F intoxication. Some studies6-9 have reported a decrease in SOD and GPx antioxidant enzyme activities in different tissues and an increase in MDA level that are indicative of lipid peroxidative damage as well as changes in membrane lipid and lipid composition due to F. The significant increases in MDA level in the liver of our sheep with fluorosis and increases in the kidneys of those sheep suggest that oxidative damage was occurring in these tissues.

There are also studies on changes in lipid metabolism from F intoxication. Vatassery et al.10 and Dousset et al.11 observed decreases in total lipid and cholesterol levels in serum and liver of pigs at the first stage of F intoxication. Hypercholesterolemia has been noted with fluorosis,30 and Mąkowska et al.12 have reported a decrease in β-lipoprotein and an increase in cholesterol levels of animals exposed to high F concentration. In contrast, Chinoy et al.31,32 did not find a significant change in lipid levels in people or in male rats at the duration of F exposure. However, serum triglyceride and cholesterol levels change as enzyme activity is altered during exposure to F, which causes inhibition of fatty acids oxidation and triglyceride lipase, some non-specific esterases, and pyrophosphatases.30 Mąsliwiec et al.33 have reported that introduction of Se in experimentally NaF-intoxicated rats decreased serum LDL-cholesterol and total lipid and triglyceride levels, but it increased HDL-cholesterol. In the present study, the concentrations of the serum lipoprotein levels of sheep with and without fluorosis showed no significant differences between the two groups. Wang et al.34 reported a sharp decline in the liver cholesterol level of rats with fluorosis, but this decrease was not statistically significant compared with that of the controls. Even though the decline was not significant, it might be an important factor to explain the functional disorders in organs and cells in the pathogenesis of chronic fluorosis in membrane lipids.

On the basis of the results observed here, we conclude that the antioxidant/pro-oxidant status of soft tissues such as kidney, liver, and muscle in the fluorosed sheep were affected by endemic F intoxication. Serum lipoprotein levels, however, were not noticeably affected by F. Further detailed studies at the molecular level, aimed at preventing and treating F intoxication, are needed because endemic fluorosis is a serious health problem for people and animals in this part of Turkey.
ACKNOWLEDGEMENT

We are grateful to the University of Yüzüncü Yıl Scientific Research Foundation for funding this project.

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