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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 Ağri 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 Ağri 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 Ağri located north of Lake Van in eastern Turkey.

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MATERIALS AND METHODS

Animal material: Ayranci 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 Ağri.

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 –80ºC. 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 levels[17] were estimated with a spectrophotometer by the color created by free-sulfhydril 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)

	Fluorosis group (n=15)	Control group (n=10)
Urinary F (ppm)	6.74±0.49*	1.50±0.30

*p<0.001 in comparison to control.

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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)

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

*$p < 0.05$ 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)

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

*$p < 0.05$ 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)

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

*$p < 0.05$ 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 5. Serum lipoprotein levels of sheep with and without fluorosis (mean±SD)

Lipoprotein	Fluorosis group (n=15)	Control group (n=10)
β-lipoprotein (LDL) %	31.09±2.02	29.53±2.53
Pre-β-lipoprotein (VLDL) %	38.81±3.40	40.90±3.94
α-Lipoprotein (HDL) %	29.21±3.79	27.84±1.47

DISCUSSION

Normal urinary F levels of sheep are reported to be 1.00–1.40 ppm.[3,4] 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.[17] Şendil and Bayşu[21] 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.[3] 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.[22] 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.[4]

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.[23] 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.[6,7,24] As a center of metabolic activity, the liver plays a key role in F metabolism. The kidney is an organ of F excretion[6] that is more subject to failure with exposure to increased levels of ingested F, leading to increased serum F levels in humans[25] and experimental animals.[26] 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.[24,27] It is well established that impairment of kidney function can occur in both chronic[6,23,27] and acute[28] 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.[6,27,28] Increased MDA (TBARS) levels in kidneys have been found as the result of F treatment, indicating lipid peroxidative damage in kidney tissue.[6,27] With elevated levels of F intake, the liver undergoes functional and structural changes.[1] 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 studies[6-9] 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] Myśliwiec 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.

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REFERENCES

1 Oktay C. Effect of high fluoride containing drinking water on skeleton and dental age. In: Seminar on "Problems of high fluoride waters;" 1977 Sept 6-10; Erzurum, Turkey.

2 Griffith-Jones W. Fluorosis in dairy cattle. Vet Rec 1977;100:84-9.

3 Ergun HS, Rüssel-Sinn HA, Baysu N, Dündar Y. Studies on the fluoride contents in water and soil, urine, bone and teeth of sheep and urine of human from eastern and western parts of Turkey. Dtsch Tierarztl Wochenschr 1987;94:416-20.

4 Fidancı UR, Baysu N, Ergun H. The fluoride content of water sources in Kızılcaoren Village in Eskisehir. Tr J Med Sci 1994;20:15-7.

5 Fidancı U, Salmanoğlu B, Maraslı S, Maraslı N. Natural and industrial fluorosis in Middle Anatolia region and its effects on animal health. Tr J Vet Anim Sci 1998;22:537-44.[in Turkish].

6 Guan ZZ, Xiao KQ, Zeng XY, Long YG, Cheng YH, Jiang SF, Wang YN. Changed cellular membrane lipid composition and lipid peroxidation of kidney in rats with chronic fluorosis. Arch Toxicol 2000;74(10):602-8.

7 Wang YN, Xiao KQ, Liu JL, Dallner G, Guan ZZ. Effect of long term fluoride exposure on lipid composition in rat liver. Toxicology 2000;146(2-3):161-9.

8 Sindelar PJ, Guan ZZ, Dallner G, Ernster L. The protective role of plasmalogens in iron-induced lipid peroxidation. Free Radic Biol Med 1999;26:318-24.

9 Machoy-Mokrzynska A, Put A, Ceglecka M, Mysliwiec Z. Influence of essential phospholipids (EPL) on selected biochemical parameters of lipid metabolism in rats chronically exposed to ammonium fluoride vapours. Fluoride 1994;27(4):201-4.

10 Vatassery GT, Ophaug RH, Singer L. The effect of fluoride intake on the total lipid, cholesterol and vitamin E levels in sera and liver of guinea pigs on high fat diet. Life Sci 1980;2:1961-6.

11 Dousset JC, Rioufol C, Philibert C, Bourbon P. Effects of inhaled HF on cholesterol, carbohydrate and tricarboxylic acid metabolism in guinea pigs. Fluoride 1987;20(3):137-41

12 Mąkowska R, Szymanska H. Cholesterol and lipid complexes in serum of chemical plant workers chronically exposed to fluoride compounds. Szczecinskie Tow Nauk Szczecin 1971;2:53-7.

13 Murray KR, Granner DK, Mayes PA, Rodwell VW, editors. Harper's Biochemistry. 21st ed. East Norwalk, CT, and San Mateo, CA: Appleton and Lange Medical Publications;1990.

14 Singer L, Armstrong WD, Vogel JJ. Determination of fluoride content of urine by electrode potential measurements. J Lab Clin Med 1969;74(2):354-8.

15 Kahraman T. The effects of electrification on rat erythrocyte and tissue anti-oxidant enzyme (superoxide dismutase, glutathion peroxidase) activities, lipid peroxidation and glutathion levels [doctoral dissertation]. Van, Turkey; Yüzüncü Yıl University; 1998.

16 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbutiric acid reaction. Anal Biochem 1979;95:351-8.

17 Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.

18 Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984;105:114-5.

19 Glutathione peroxidase and superoxide dismutase commercial kit. Crumlin, Co. Antrim, Northern Ireland, UK: Randox Laboratories Ltd; 1997.

20 Helena lipoprotein electrophoresis procedure. Beaumont, TX, USA: Helena Laboratories; 1998.

21 Sendil C, Baysu N. Fluorosis intoxication in human and animals observed in villages of Doğubeyazıt district of Ağrı province and first presentation about presence of this problem in villages of Muradiye district of Van province. Vet J Ankara Univ [Ankara Üniversitesi Veteriner Fakültesi Dergisi] 1974;10:474-89.

22 Altıntaş A, Fidancı UR, Sel T, Duru Ö, Başsatan A. Serum proteins electrophoresis and kidney function in sheep with natural and industrial fluorosis. Vet J Ankara Univ [Ankara Üniversitesi Veteriner Fakültesi Dergisi] 2000;47(2):105-14.

23 Kessabi M, Hamliri A, Braun JP. Experimental fluororsis in sheep: Alleviating effects of aluminum. Vet Hum Toxicol 1986;28(4):300-4.

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24 Akdoğan M, Bilgili A, Karaöz E, Gökçimen A, Eraslan G, Üstüner E. The structural and biochemical changes of kidney tissue on fluorosis in rabbits. Turk J Vet Anim Sci 2002;26(1):71-7

25 Parsons V, Choudhury AA, Wass JA, Vernon A. Renal excretion of fluoride in renal failure and after renal transplantation. Br Med J 1975;1:128-30.

26 Turner CH, Owan I, Brizendine EJ, Zhang W, Wilson ME, Dunipace AJ. High fluoride intakes cause osteomalacia and diminished bone strength in rats with renal deficiency. Bone. 1996;19(6):595-601.

27 Singh PP, Barjatiya MK, Dhing S, Bhatnagar R, Kothari S, Dhar V. Evidence suggesting that high intake of fluoride provokes nephrolithiasis in tribal populations. Urol Res 2001;29(4):238-44.

28 Usuda K, Kono K, Dote T, Nishiura K, Tagawa T. Usefulness of the assessment of urinary enzyme leakage in monitoring acute fluoride nephrotoxicity. Arch Toxicol 1999;73(6):346-51.

29 Burdon RH. Free radicals and cell proliferation. In: CA Rice-Evans CA, Burdon RH, editors. Free radical damage and its control. Bernardi G, series editor. New Comprehensive Biochemistry. Vol. 28. London, Amsterdam, New York: Elsevier Science; 1994. pp.155-89.

30 Czerny BA, Put A, Myśliwiec Z, Juzyszyn Z. The influence of quercetin on some biochemical parameters in rats exposed to environmental contamination with fluorine compounds. Polish Journal of Environmental Studies 2000;9(3):157-61.

31 Chinoy NJ, Narayana MV, Sequeira E, Joshi SM, Barot JM, Purohit RM, et al. Studies on effects of fluoride in 36 villages of Mehsana District, North Gujarat. Fluoride 1992;25(3):101-10.

32 Chinoy NJ, Sharma M, Michael M. Beneficial effects of ascorbic acid and calcium on reversal of fluoride toxicity in male rats. Fluoride 1993;26(1):45-56.

33 Myśliwiec Z, Machoy-Mokrzynska A, Juzyszyn Z, Czerny B, Put A. Effects of selenium on serum lipids and enzyme activities in fluoride-intoxicated rats. Fluoride 2002;35(3),168-75.

34 Wang YN, Xiao KQ, Liu JL, Dallner G, Guan ZZ. Effect of long term fluoride exposure on lipid composition in rat liver. Toxicology 2000;146 (2-3):161-9.