

CHANGES IN ERYTHROCYTE PARAMETERS OF FLUOROTIC SHEEP

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SUMMARY: The aim of this study was to investigate changes in oxidative stress parameters of erythrocytes in twenty 3–4 year-old fluorotic sheep as compared to twenty controls. Malondialdehyde (MDA) levels (mean \pm SD) were significantly elevated in erythrocytes of the fluorotic sheep (2.51 ± 0.16 nmol/mL) in comparison to the control sheep (1.32 ± 0.13 nmol/mL). Erythrocyte $\text{Na}^+\text{-K}^+$ adenosine-5'-triphosphatase ($\text{Na}^+\text{-K}^+$ ATPase) activity in the fluorotic sheep (0.003 ± 0.0005 $\mu\text{mol Pi/mg protein/h}$) was significantly lower than in the controls (0.005 ± 0.0005 $\mu\text{mol Pi/mg protein/h}$), as was the activity of glucose-6-phosphate dehydrogenase (G6PD) (510 ± 23.30 mU/g Hb) compared to the controls (1418 ± 85.40 mU/g Hb). The results are explained by fluoride-induced oxidative stress reflected in elevated erythrocyte MDA levels that cause decreased enzyme activity of $\text{Na}^+\text{-K}^+$ ATPase and G6PD by affecting membrane structure.

Keywords: Fluorotic sheep; Glucose-6-phosphate dehydrogenase; Malondialdehyde; $\text{Na}^+\text{-K}^+$ ATPase; Sheep fluorosis.

INTRODUCTION

Present as a trace element in various amounts in food, air, and water, fluorine (as fluoride ion) plays an important role in biological functions that alter teeth and bones. In elevated concentrations, it can be very noxious in the environment, affecting the health of humans and animals as well as plant life. Two patterns of fluoride toxicity are well known: endemic fluorosis and industrial fluorosis. Endemic fluorosis is caused primarily by high concentrations of fluoride in drinking water¹ and/or by fluoride in food, especially from domestic use of high-fluoride coal,² while industrial fluorosis is due mainly to fluoride air pollution. However, the toxicity kinetics and pathogenesis of fluoride on the whole body are unclear.

Chronic fluoride intoxication, or fluorosis, is a worldwide health problem and is endemic in areas where the fluoride content of drinking waters is high. Its primary manifestations in humans and mammals are mottling of teeth and osteosclerosis of the skeleton.³ In the eastern part of Turkey, fluorosis is a serious health problem for humans and animals. They consume fluoride from water near the volcanic areas of Mt. Ararat and Mt. Tendürek. Human tooth and bone deformities are widely observed in these areas.⁴ In Isparta, more than 50 per cent of the districts have high fluoride (F) levels (4.03 ppm) in their drinking waters.⁴ In Ağrı (Dogubeyazıt), levels of 12.5 ppm F have been reported, and, in Van (Muradiye), levels of 5.7–15.2 ppm F are on record.⁴ Fluoride concentrations of 8.1 ppm have been found in

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sheep urine, and 4.3 ppm in human urine. In Eastern Anatolia, 3374–5149 ppm have been reported in sheep bone ash, and concentrations of 3787–5299 ppm were found in their teeth.⁴

The aim of this study was to investigate the changes in erythrocyte malondialdehyde (MDA) levels, and the activities of Na⁺-K⁺ adenosine triphosphatase (Na⁺-K⁺ ATPase) and glucose-6-phosphate dehydrogenase (G6PD) in fluorotic sheep.

MATERIALS AND METHODS

As living research materials, samples from twenty 3–4 year-old fluorotic and twenty healthy Morkaraman Sheep were examined. Fluoride ion concentration in urine was determined by a specific fluoride electrode.⁵

Lipid peroxidation in erythrocytes was assessed by determination of MDA by the method of Akkus.⁶

Na⁺-K⁺ ATPase activity in erythrocytes was measured as the release of inorganic phosphate from hydrolysis of ATP (adenosine triphosphate) in the presence and absence of ouabain. Erythrocytes were incubated at 37° C for 60 min in 1 mL of a solution containing 3 mM ATP (pH 7.0), 50 mM NaCl, 20 mM KCl, 3 mM MgCl₂, and 100 mM tris-HCl buffer (pH 7.4). To inhibit Na⁺-K⁺ ATPase activity, 1 mM ouabain was added. The reaction was stopped by the addition of trichloroacetic acid. ATPase activity was expressed as nanomoles of phosphorus released per mg protein/h.^{7,8}

Glucose-6-phosphate dehydrogenase (G6PD) activity was estimated on hemolysates by the use of a commercially available kit (Randox Lab.).⁹

Statistical significance of the results was assessed by the Student t-test.

RESULTS

The Table below records the results of the urinary fluoride determinations and the analyses for the erythrocyte levels of MDA and the activities of Na⁺-K⁺ATPase and G6PD in both control and exposed sheep. The activities of Na⁺-K⁺ATPase and G6PD were significantly lowered, while the MDA levels were increased in erythrocytes of fluorotic sheep compared to those of control sheep (p<0.001).

Table. Urinary fluoride and erythrocyte levels of MDA and activities of Na⁺-K⁺ ATPase and G6PD in two groups of sheep

	Control group (n=20)	Exposed group (n=20)
Urinary fluoride (ppm)	1.65 ± 0.35	23.84 ± 4.74*
MDA (nmol/mL)	1.32 ± 0.13	2.51 ± 0.16*
Na ⁺ -K ⁺ ATPase (μmol Pi/mg protein/h)	0.005 ± 0.0005	0.003 ± 0.0005*
G6PD (mU/g Hb)	1418 ± 85.40	510 ± 23.30*

^aValues are mean ± SD. *p< 0.001.

DISCUSSION

It is important to understand fluorosis at biochemical and molecular levels. Many researchers have reported biochemical changes in the composition of bone, urine, plasma, and the levels of some hormones.¹⁰⁻¹² Fluoride has been shown to inhibit many enzymes involved in the pentose phosphate pathway, antioxidant defense systems, and in myosin-ATPase activity.^{13,14}

Chronic degenerative diseases including chronic fluorosis have been associated with increased production of reactive oxygen species, and lipid peroxidation. But the pathogenesis of chronic fluorosis is poorly understood.^{11,12}

Metabolic imbalances, oxidative stress, and lipid peroxidation are evidently involved in the pathogenesis of chronic fluorosis, but the results of many studies are conflicting and contradictory to one another. Decreases in the activity of SOD (superoxide dismutase) and GPx (glutathione peroxidase) have been found in people living in endemic fluorosis areas.¹ Shivarajashankara *et al*¹⁵ reported that, in children aged 3 to 10 years with endemic fluorosis, erythrocyte MDA and ascorbic acid levels and GPx activity were increased, while GSH (glutathione) levels were decreased. They also found that fluoride intoxicated rats exhibited increased MDA, ascorbic acid, and GSH levels and GPx activity in their red blood cells.¹⁶ In view of such findings it has been suggested that there is increased oxidative stress in skeletal fluorosis.

On the other hand, Reddy *et al*¹⁷ reported that they did not find significant changes in any of the antioxidant parameters tested, including ascorbic acid, GSH, catalase, SOD, GPx, and GST (glutathione S-transferase) in fluorotic humans and rabbits. By contrast, in another study,¹⁸ it was reported that fluoride injection into rats for 14 days at 20 mg/kg body weight/day/ip caused decreases in GST, SOD, and catalase activity in the brain. Similar results in rats from fluoride in their drinking water have also been reported.¹⁹

In our study, we have found that erythrocyte MDA levels were increased significantly, but Na⁺-K⁺ ATPase and G6PD activities were significantly decreased ($p < 0.001$) in fluorotic sheep. Increased MDA concentration is correlated with enhanced lipid peroxidation in fluorotic animals.

Na⁺-K⁺ ATPase is a heterodynamic plasma membrane protein responsible for cellular ionic homeostasis in almost all animal cells. It mediates coupled transport of Na and K ions and contributes to the maintenance of electrochemical gradient of the cell.²⁰ In our fluorotic sheep erythrocyte Na⁺-K⁺ ATPase activity was found to be decreased, compared with the control group. This could be associated with peroxidations of membrane phospholipids and the accumulation of MDA, which inhibits the activity of membrane Na⁺-K⁺ ATPase activity.²¹

Han Bo *et al*²² studied 32 cows selected for grazing on high fluoride forage and found inhibition of Na⁺-K⁺ ATPase activity as well as immune system and hemopoietic dysfunctions. Moreover, free radical and MDA levels were higher, but whole blood GPx, CAT (catalase), and SOD activities were lower than those in the control group.

We also observed that the erythrocyte G6PD activity of fluorotic sheep was decreased. The low activity of G6PD may have resulted from alterations in protein structure of the enzyme by the action of free radicals. A G6PD deficiency is usually well tolerated except for hemolytic crisis associated with oxidative stress.²³ Moreover, carbohydrate metabolism disturbances are also seen in fluorosis. In fluorotic rats the activity of liver G6PD is decreased and glycogen turnover depressed.²⁴

In conclusion, our findings in sheep indicate that chronic fluorosis affects lipid peroxidation and causes oxidative stress manifested by increased erythrocyte levels of MDA and decreased activities of Na⁺-K⁺ ATPase and G6PD.

REFERENCES

- 1 Li J, Cao S. Editorial: Recent studies on endemic fluorosis in China. *Fluoride* 1994;27:125-8.
- 2 Wei Z. Endemic food-borne fluorosis in Guizhou, China. *Prev Med J* 1979;13:148-51.
- 3 Yasar S. Investigation of the vitamins and mineral levels in sheep with fluorosis. Master thesis, Y Y University Health Science Institute, Van, Turkey; 2003.
- 4 Ergun H, Russel H, 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 Tieraztl Wschr* 1987;94:416-20.
- 5 Singer L, Armstrong WD, Vogel JJ. Determination of fluoride content of urine by electrode potential measurements. *J Lab Clin Med* 1969;74:354-58.
- 6 Akkus I. Free radicals and their pathophysiological effects. Konya, Turkey: Mimoza Press; 1995.
- 7 Serpersu E, Ciliv G. Some properties of Na-K dependent adenosine triphosphate from human erythrocyte. *Biochem Med* 1978;20:31-9.
- 8 Bildik A, Belge F, Yur F, Alkan M, Kilicalp D. The effect of hyperthyroidism on the levels of Na⁺K⁺ATPase, G6PD and glutathione. *Israel J Vet Med* 2002;57:19-22.
- 9 Glucose-6-Phosphate Dehydrogenase test for in vitro diagnostic use. Randox Laboratories Ltd. United Kingdom. 1997.
- 10 Dogan I. Investigation of antioxidant compounds of fluorotic sheep. Master thesis, Y Y University Health Science Institute, Van, Turkey; 2002.
- 11 Das AA. Fluorosis. In: Bamji MS, Rao NP, Reddy V, editors. *Textbook of human nutrition*. New Delhi: Oxford & IBH Publishing; 1996. p 424-40.
- 12 Krishnamachari KA. Skeletal fluorosis in humans: A review of recent progress in the understanding of the disease. *Prog Food Nutr Sci* 1986;10:279-314.

- 13 Carslon JR, Suttie JW. Pentose phosphate pathway enzymes and glucose oxidation in fluoride-fed rats. *Am J Physiol* 1966;210:79-83.
- 14 Park S, Ajtai K, Burghardt P. Inhibition of myosin ATPase by metal fluoride complexes. *Biochim Biophys Acta* 1999;1430:127-40.
- 15 Shivarajashankara YM, Shivashankara AR, Rao SH, Bhat GP. Oxidative stress in children with endemic skeletal fluorosis. *Fluoride* 2001;34:103-7.
- 16 Shivarajashankara YM, Shivashankara AR, Bhat GP, Rao SH. Effect of fluoride intoxication on lipid peroxidation and antioxidant systems in rats. *Fluoride* 2001;34:108-13.
- 17 Reddy GB, Khandare AL, Reddy PY, Rao GS, Balakrishna N, Srivalli I. Antioxidant defense system and lipid peroxidation in patients with skeletal fluorosis and in fluoride-intoxicated rabbits. *Toxicol Sci* 2003;72:363-8.
- 18 Vani ML, Reddy KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride* 2000;33:17-26.
- 19 Shivarajashankara YM, Shivashankara AR, Bhat PG, Rao SH. Brain lipid peroxidation and antioxidant systems of young rats in chronic fluoride intoxication. *Fluoride* 2002;35:197-203.
- 20 Palma F, Ligi F, Soverchia C, Fioritti A. HPLC method for measuring Na⁺-K⁺ATPase and Ca⁺⁺-Mg⁺⁺ATPase in erythrocytes from different species of mammals. *Boll Soc Ital Biol Sper* 1991;67:759-66.
- 21 Uysal M. Erythrocyte lipid peroxidation and (Na⁺-K⁺) ATPase activity in cholesterol fed rabbits. *Internat J Vit Nutr Res* 1986;56:307-10.
- 22 Han Bo, Manyu L, Yan S. Studies on the toxicology of endemic fluorosis in cattle. Abstracts XXII World Buiatrics Congress, Hannover, Germany, August 2002;18-23.
- 23 Alfinito F, Calabro V, Cappellini M, Rotoli B, Luzzato L. Glucose-6-phosphate dehydrogenase deficiency and red cell membrane defects: additive or synergistic interaction in producing chronic hemolytic anemia. *Br J Haematol* 1994;87:148-52.
- 24 Underwood EJ. Trace Elements in Human and Animal Nutrition. Fourth Edition. New York and London: Academic Press Inc; 1977; p 363.