

INVESTIGATION OF ANTIOXIDANT COMPOUNDS IN FLUOROTIC SHEEP

Inci DOGAN¹, Handan MERT^{1*}, Kivanc IRAK², Nihat MERT¹

¹Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Biochemistry,
Van, Turkey

²University of Siirt, Faculty of Veterinary Medicine, Department of Biochemistry, Siirt, Turkey

Corresponding author email: hg8803@hotmail.com

Abstract

Fluorosis, a condition which usually affects the formation of bone and teeth in human and animals, is an important health problem in Van and Agri provinces. This study was performed to determine the levels and the changes of antioxidant compounds in fluorotic sheep. 30 fluorotic sheep and 20 healthy Morkaraman sheep of 3-4 years old were used as living research materials. The diagnosis of fluorosis was confirmed by clinical examinations. The urine fluoride level was determined. Blood of all animals was taken from vena jugularis by appropriate techniques and analyzed for glutathione peroxidase (GPx), glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), sialic acid (SA) and lipid-bound sialic acid (LSA). The levels of these parameters in healthy and fluorotic group were: 1028-416.8 mU/ml, 23.23-50.16 mg/dl, 9.25- 7.88 mU/ml, 1.62-0.56 nmol/ml, 51.19-46.33 mg/dl and 9.77- 12.16 mg/dl, respectively. Urine fluoride (F) levels were 1.65 ppm in healthy and 23.84 ppm in fluorotic sheep groups. Statistical differences was found between the average values of healthy and fluorotic groups as $p \leq 0.0001$ in GPx and GSH, $p \leq 0.001$ in MDA, $p \leq 0.05$ in SA, $p \leq 0.01$ in LSA and $p \leq 0.001$ in urine F levels. No statistical differences were found in SOD levels. ($p \geq 0.05$) The results obtained in this study indicate that important changes were found in antioxidant systems of fluorotic sheeps.

Key words: antioxidant, blood, fluorosis, sheep, urine.

INTRODUCTION

Fluorosis is a result of excessive fluoride (F) intake for a prolonged time and occurs in two different forms as acute and chronic. Ionic F in plasma increases and F accumulates in the body. Various changes occur after chronic administration of F in blood, brain, liver and musculoskeletal system in human and animals. Excess F intake can inhibit the activity of many enzymes. Chronic fluorosis can be commonly observed in industrial areas, volcanic and tectonic areas. The Eastern part of Turkey has volcanic structure eruption of volcano enriched soil, with ground and underground water contaminated with F. The high level of F can be dangerous to all living creatures in this area. Especially Muradiye, Caldiran, Tendurek and Mt. Agri had been severely affected. The levels of F have been found elevated in plant, soil and biological samples from animal and human (Underwood, 1966; Jones 1977; Shupe et al., 1984; Ergun et al., 1987; Walton, 1988). Antioxidants defense system inhibits the free radical production functions for the scavenger of free radicals, repair of cells, increase the

capacity of antioxidant stops the secondary chain reactions. Superoxide dismutase, glutathione peroxidase, tocopherols, glutathione, retinol, ascorbic acid are taken into account for antioxidant compounds. Many studies indicate that generation of lipid peroxidation product, free radicals and altered antioxidant defense systems are closely related with fluorosis (Han et al., 2000; Freemann and Cropp, 1982).

Sialic acid is a monosaccharide with nine carbon backbone and also name for N-acetylneuraminic acid (NANA) which is widely distributed in animal tissue, especially in glycoproteins, mucoproteins, glycolipids and gangliosides. Sialic acid also contributes to creating negative charge on the cell surface. SA reduction were observed in some chronic diseases which causes to produce excess glycoprotein. Metastatic cancer cells often have sialic acid rich glycoprotein (Dnistrian et al., 1982; Traving and Schauer, 1998).

There are many researches on the F level of the biological material such as teeth, urine, bone, milk or blood, but limited study has been done on the antioxidant levels of fluorotic sheep.

The study was designed to investigate the effect of F on antioxidant systems. For this purpose the levels of F in the urine, the amounts of glutathione, SA, LSA, MDA and the activities of GPx, SOD in blood have been determined in healthy and fluorotic sheep.

MATERIALS AND METHODS

Thirty chronic fluorotic sheep raised in Van and surrounding villages of Caldıran and twenty healthy sheep from the Research Farm of Faculty of Veterinary Medicine in Van were used as living research materials.

The urine samples from all sheep were analyzed for F amount by fluorimeters (Singer et al., 1969)

Blood samples were taken from v. jugularis, centrifuged at 3000 rpm for 15 minutes and plasma were separated into erythrocytes and washed three times with 0.9 % NaCl at 2000 rpm for 8 minutes. The washed erythrocytes were transferred to Eppendorf tubes. The erythrocyte package was used to determine the activity of GPx and SOD enzymes using Calbiochem enzyme kit assay (Ransod Superoxide Dismutase Enzyme Kit, Ransel Glutathione Peroxidase Enzyme Kit).

GSH levels were determined in whole blood spectrophotometrically using Ellman's Reagent at 412 nm (Beutler et al., 1963). MDA levels were measured in whole blood spectrophotometrically according to color formation with thiobarbituric acid at 412 nm. (Gutteridge, 1995; Sushil et al., 1989).

Blood samples taken into tubes without anticoagulant were centrifuged at 2500 rpm for 10 minutes and sera was separated.

The serum SA were determined spectrophotometrically using Erlich reagent comparing the reading obtained at 525 nm to a standard curve developed from a known sample of N-acetylneuraminic acid (NANA) under the same conditions (Gerbaut et al., 1973).

LSA levels were also determined spectrophotometrically using resorcinol solution then reading at 580 nm the extracted blue color present in the organic layer, determining the amount of lipid bound sialic acid by comparing the reading obtained at 580 nm to a standard curve developed from a known sample of N-acetylneuraminic acid

(NANA) under the same conditions with a special formula (Dnistrian et al., 1982; Katopodis et al., 1982).

All data were statistically evaluated using unpaired t-test.

RESULTS

All the average values of analyzed parameters were shown in Table 1. The differences between averages of two groups were analyzed and statistical importance was found as F levels ($p \leq 0.001$), activity of GPx ($p \leq 0.0001$), GSH ($p \leq 0.0001$), in MDA ($p \leq 0.001$), SA ($p \leq 0.05$) and LSA ($p \leq 0.01$).

Table 1. Biochemical parameters of urine and blood of healthy and fluorotic sheep

Parameters	n	Control X \pm SEM	n	Fluorotic Sheep X \pm SEM	T	p
Fluoride (urine) (ppm)	10	1.65 \pm 0.35	18	23.84 \pm 4.74	4.672	***
Glutathione peroxidase (mU/ml)	20	1028 \pm 80.92	28	416.8 \pm 25.06	7.214	****
Glutathione (mg/dl)	20	23.23 \pm 0.75	30	50.16 \pm 4.22	6.285	****
Superoxide dismutase (U/ml)	19	9.25 \pm 0.88	30	7.88 \pm 0.45	1.383	NS
Malondialdehyde (nmol/ml)	20	1.62 \pm 0.24	30	0.56 \pm 0.12	3.980	***
Sialic acid (mg/dl)	20	51.19 \pm 1.81	27	46.33 \pm 1.65	1.967	*
Lipid-bound sialic acid (mg/dl)	20	9.77 \pm 0.54	27	12.16 \pm 0.57	2.947	**

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, NS non-significant

DISCUSSIONS

As of the geological structure, Eastern Anatolia is young and has high volcanic activity. Fluorosis has been determined in people and animals living in this region. High fluoride levels were determined in drinking waters. Consuming the plant and water with high amount F is the main causes of fluorosis. (Dobaradaran et al., 2008a; Dobaradaran et al., 2008b)

Fluoride can be excreted from body by kidney therefore, the measurement of F in urine is used to diagnose the fluorosis. Ergun et al. (1987) had reported a urine F levels of 8.11-1.49 ppm, in sheep raised in the Eastern and respectively

Eagen region. Şendil and Bayşu (1973) determined the sheep urine F levels between 3.80-30.61 and 3.80-26.61 ppm in Muradiye and Dogubeyazit town. In the present study the amount of urine F of the control and fluorotic sheep were found as 1.65-23.84 ppm, respectively ($p \leq 0.001$) and supported the endemic and extensive fluorosis in this area.

Chinoy and Dipti (2000) studied the effects of protein on fluorosis. They added 5-10-20 mg/kg NaF (Sodium fluoride) to the protein-deficient diets of rats and observed that the activity of liver GSH, SOD and CAT were decreased and lipid peroxidation was increased. Rats feed with added protein diets didn't show any significant changes. The authors concluded that protein addition prevented the F intoxication.

Shivarajashankara et al. (2001) feed Wistar albino rats with 100 ppm added NaF for 4 months and analyzed the parameters of antioxidants system. While the activities of GPx, erythrocyte GSH, and ascorbic acid levels were increased, SOD and uric acid levels were decreased.

Han et al. (2000) investigated the antioxidant enzymes, lipid peroxidation and free radicals levels in low Se and Cu condition with endemic fluorosis in cattle. ROS and MDA levels in fluorosis group were increased, while GPx and erythrocyte SOD levels were decreased. They concluded that high amounts of F affected the antioxidant system, and decreases the lipid peroxidation LPO and ROS levels or free radical metabolic imbalance and antioxidation disorder were the main factors causing endemic fluorosis.

In the present study the levels of GSH and GPx in fluorotic and healthy sheep were found as 50.16-23.23 mg/dl ($p \leq 0.001$), and 416.8-1028 mU/ml ($p \leq 0.001$), respectively. Both parameters were significantly changed because of the oxidative stress in fluorotic sheep.

The function of SOD enzymes is to eliminate superoxide radicals in the body. The activity of SOD enzymes in fluorotic and healthy sheep were 7.88-9.25 U/ml respectively. The differences between groups were not significantly important. Wu et al. (2015) have studied on the oxidative stress of bone tissue in rats with chronic fluorosis treated with antioxidant, the oxidative damage of lipid, protein

and DNA. They concluded that the activity of SOD and CAT of tissues are inhibited and suppression function of hydroxy free radical is decreasing under fluorosis influence, which results in protein damage. Oxidative stress is considered to be one of the mechanisms of skeletal fluorosis.

Animal species, environmental conditions, F intake by food and water, time and amount of F intake, the antagonistic compounds with F in the medium, the age of the animals are considered significant factors in the development of symptoms of fluorosis and effects of F on animals. Some animals can have adaptive phase as reported by Shivarajashankara et al. (2001). They reported that adaptive changes could be occurred in the antioxidant system of blood, brain, liver, and protection mechanism can be formed against the oxidative stress of F intoxication.

The lipid peroxidation product, malondialdehyde, must be determined in chronic diseases. In subclinical mastitis the MDA level was increased as a result of oxidative stress (Gutteridge, 1995). Güven, and Kaya (2005) have studied the effect of fluorosis on blood malondialdehyde (MDA) and reduced glutathione (GSH) levels in yearling Tuj ewe-lamb. Lipid peroxidation and GSH levels in the fluorotic group were significantly higher than controls (MDA $p < 0.01$, GSH $p < 0.05$). They concluded that fluorine may cause an increase in lipid peroxidation in cases of fluorosis. In this study MDA levels in healthy and fluorotic sheep were 1.62-0.56 nmol/ml ($p \leq 0.001$). Fluorosis generally stimulates the formation of ROS and increases lipid peroxidation. In contrast to this statement, here, low MDA levels were determined in fluorotic group than the controls. MDA could be a sign for the low lipid peroxidation in our cases.

Yur et al (2013) found that 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. They concluded that different degrees in the pro-oxidant/antioxidant status of soft tissues such as kidney, liver, and muscle were affected by F intoxication.

Sialic acid is mainly located in the cell, gangliocyte membrane and in glycoproteins. The SA increases in some chronic disease such

as tuberculosis, liver cirrhosis, chronic bovine hematuria, lymphatic leukemia and some parasitic diseases were reported by (Freeman and Cropro, 1982; Traving and Schauer, 1998). The sialic acid and lipid-bound sialic acid levels of fluorotic and healthy sheep were found as 46.33-51.19 mg/dl ($p \leq 0.05$) and 12.16-9.77 mg/dl ($p \leq 0.01$), respectively. Increases of LSA could be caused by the inhibitory effect of F on aerobic glycolysis enzymes and possible destruction and degeneration of erythrocyte membrane.

CONCLUSIONS

As conclusion, fluorosis was detected in sheep raised in the Van and Agri provinces and surrounding villages. The levels of GSH, LSA were increased and SA, GPx, SOD and MDA levels were decreased in the blood of fluorotic sheep. These changes could be the result of oxidative stress of fluorosis and these changes must be considered during clinical and scientific studies.

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