THE DISTRIBUTION OF MINERALS IN SOME TISSUES OF SHEEP WITH FLUOROSIS

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SUMMARY: The aim of this study was to investigate the metabolism of certain minerals (calcium, copper, magnesium, manganese, phosphorus, and zinc) in bone, tooth, and gum tissues in sheep with fluorosis. Ten sheep with fluorosis were compared to 10 non-fluorotic control sheep. In the bone, in the fluorosis group, the level of copper decreased significantly (p<0.03) while the levels of the other minerals were unchanged. In the teeth, in the fluorosis group, the level of calcium increased (p<0.01) and decreases occurred in the levels of magnesium (p<0.05), zinc (p<0.05) and manganese (p<0.01). In the gums, in the fluorosis group, the calcium and manganese levels were significantly higher (p<0.01) and the magnesium level was significantly lower (p<0.05). No differences were found in the levels of phosphorus in the bone, tooth, or gum tissues. In conclusion, the bone, tooth, and gum tissue levels of several minerals with important structural or trace element catalytic functions, were found to be altered in sheep affected by chronic fluorosis.

Key Words: Bone; Calcium; Copper; Fluorosis; Gum; Macro elements; Magnesium; Manganese; Phosphorus; Sheep; Tooth; Trace elements; Zinc.

INTRODUCTION

This report gives further information on a group of sheep in eastern Turkey on whom serum lipoprotein and tissue antioxidant level data have been published.1

Fluoride is not an essential trace element and is not necessary for the formation of healthy bone and teeth. Excessive fluoride exposure leads to the chronic condition of fluorosis which is common in some regions of Turkey. With excessive fluoride exposure, up until the age of approximately six years, children may develop dental fluorosis with tooth discolouration and lower limb deformities may also occur.2 Adults may develop symptoms affecting many organ systems including skeletal fluorosis with pain, rigidity, and restricted movement of the cervical and lumbar spine, pelvic girdle and the knee and shoulder joints.2-14

Fluoride interacts with other minerals including trace elements. Excessive fluoride consumption reduces calcium absorption and causes calcium ions to be mobilized from bones and teeth.14-17 The serum magnesium levels rise in fluorotic sheep18 and excessive fluoride intake has effects on the zinc concentrations in almost all tissues and organs, especially in the blood.19,20 Decreases in the serum copper levels occur in fluorotic sheep19 and liver and bone copper levels are significantly reduced in fluorotic rats.21-23 Bone manganese levels rise significantly in fluorotic rats,24 while the liver and kidney manganese levels fall.23

Minerals are important for many metabolic activities and studying their interaction with fluoride may contribute to the understanding of the biological and genetic aspects of chronic fluoride toxicity. The present study was planned to

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investigate the effects of fluoride on the levels of macro and micro elements in bones, teeth and gums.

**MATERIALS AND METHODS**

*Study groups:* The 10, two-year-old or older, Akkaraman fluorotic sheep studied were among those reported on in a previous study on serum lipoprotein and tissue antioxidant levels in sheep with fluorosis. They were from the Ayrancı village in Dogubeyazit, Ağrı, an endemic fluorosis area in east Turkey. Ten control 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.

*Clinical examination:* To determine the presence of fluorosis an anamnesis was taken from the animal owners and signs of fluorosis looked for in a clinical examination of the teeth and joints.

*Analyzing of urinary fluoride levels:* The urine samples were taken from the animals and put into polyethylene tubes. The levels of urinary fluoride were measured with ion-selective electrodes and with an ionometer of WTW PH / ION 738 brand.

*Preparation of tissues:* Tissue samples (bone, tooth and gum) were collected from the sheep. A sample was taken from the end part of the front incisor teeth. Gums around these teeth were taken out by a lancet. The samples were prepared with 1% Triton-100 and mixture of nitric acid/perchloric acid (1/5) washing technique.

*Mineral analysis:* The calcium, phosphorus, and magnesium concentrations in the bone, tooth and tooth gum tissue extracts were measured at a wavelength of 5.52 nanometers in an autoanalyzer (Roche, Cobas Integre 400). The zinc, copper, and manganese concentrations were measured using an atomic absorption spectrophotometer (Unicam 929-UK) and appropriate lambs.

*Statistical Analysis:* The values obtained from all the analyses were calculated as mean± SD and evaluated by a paired samples t-test.

**RESULTS**

In the sheep with fluorosis, the copper levels were significantly lower (p<0.01) than in the control group (Table 1).

In the fluorotic sheep, the tooth tissue level of calcium was higher than in the control group (p<0.05) while the levels were lower for magnesium (p<0.05), zinc (p<0.05), and manganese (p<0.05, Table 2).

In the fluorotic animals, increases occurred in the gum tissue levels of calcium (p<0.01) and manganese (p<0.01) while the magnesium level decreased (p<0.05, Table 3).
DISCUSSION

No previous studies were found on the calcium and phosphorus levels in gum tissue but their levels in bones and teeth have been investigated. In experimental fluorotic rats, calcium increased in the forearms and some other parts of the skeleton while remaining unchanged in the femur.

In the present study, the calcium levels of bone did not change but they increased significantly in the teeth and gums of the fluorotic sheep. Yasar and Yur reported...
that the serum calcium did not change in sheep with endemic fluorosis. In contrast, Celikler\textsuperscript{19} found that fluoride increased the serum calcium in sheep with experimental chronic fluorosis. Jawed et al. found that patients with dental caries had significantly decreased levels of calcium and phosphate.\textsuperscript{13} Increased serum phosphorous levels and decreased calcium levels were observed in rats with experimental fluorosis who showed, with electron microscopy, flattened bone cells with damaged nuclei, endoplasmic reticulum, and enlarged lacunae.\textsuperscript{14}

Fluoride exposure may cause kidney damage and chronic renal disease may effect the oral health and skeletal development.\textsuperscript{32,33} Calcium forms complexes of low solubility with fluoride ions and thus reducing its absorption and resulting in calcium ions being mobilized from bones and teeth.\textsuperscript{15-17} In the present study, the bone calcium level did not change significantly and the significant accumulation of calcium in the teeth and gums followed prolonged exposure to an increased intake of fluoride. We found no difference between the fluorotic and control groups in the phosphorus levels in the bone, teeth, and gums.

Magnesium forms complexes of low solubility with fluoride thus reducing the absorption of fluoride from the gastrointestinal tract.\textsuperscript{15-17,34} Fluoride toxicity may play a key role in acute magnesium deficiency.\textsuperscript{35} Fluoride increases the serum magnesium in sheep.\textsuperscript{19} Fluoride intake of adult animals results in increased skeletal magnesium levels.\textsuperscript{36} The levels of fluoride and magnesium were found to be significantly lower in teeth with dentine erosion.\textsuperscript{37} In the present study, the bone magnesium levels did not change, but the teeth and gum magnesium levels were significantly lower in the fluorosis group. The decreases may be due to reduced magnesium absorption following its forming relatively insoluble complexes with fluoride.

Variable results have been reported for zinc levels in fluorosis with decreases being reported in serum,\textsuperscript{18} bone,\textsuperscript{20} liver,\textsuperscript{20,22} testis,\textsuperscript{22} plasma,\textsuperscript{22} and kidney,\textsuperscript{22} and increases in bone\textsuperscript{22} and kidney.\textsuperscript{21} In the present study the tooth zinc level decreased with no change being found in bone or gum tissues.

Fluoride has been found to reduce copper levels in rats in bones and liver\textsuperscript{22,23} and in sheep in serum\textsuperscript{19} Ceruloplasmin (a copper-carrying protein in blood) decreases and this might be useful in the early diagnosis of fluorosis.\textsuperscript{38} The lower bone copper level in bone found in fluorotic sheep in the present study is consistent with the literature.

Bone manganese concentrations rise significantly in rat tissues with fluorosis.\textsuperscript{20,21,23,24} In the present study, the bone manganese level was unchanged, the tooth level decreased, and the gum level increased. The accumulation of manganese in the gum tissue may have been due to the general effect of fluoride intoxication or an increase in the activity in the tooth gum tissues of various enzymes for which manganese was a cofactor.

The distribution reported in the present study of various minerals, with important structural or catalytic functions, in hard tissues like bones and teeth and soft
tissues like tooth gums, provides baseline information on the effects on these organs of chronic fluorosis and lays a foundation for future research.

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