THE RELATIONSHIP BETWEEN THE LEVELS OF THE FLUORIDE ION AND THE THYROID HORMONES IN GOATS WITH FLUOROSIS

Sezai Arslan,[a,*] İhsan Keleş[b]

Tekirdağ and Kayseri, Turkey

ABSTRACT: In the present study, 104 goats with endemic fluorosis, living at the foot of the mountains Ağrı (Mount Ararat) and Tendürek, were compared with 30 healthy goats to investigate the relationship between the plasma levels of the fluoride ion (F) and the thyroid hormones. In addition to the 104 goats with fluorosis all having dental fluorosis, 1.92% had exostoses in their palpable bones, 19.23% had mastication difficulties with weight loss, 2.88% had nail deformities, and 7.69% had lameness. However, signs of goitre in their necks were not present on inspection and palpation. The plasma F concentration was significantly higher ($p<0.001$) in the fluorosis group compared to the control group. The plasma F concentrations tended to be higher in the older animals compared to the younger animals, but not significantly so. Compared to the control group, the serum total thyroxine (TT_4) was significantly reduced ($p<0.01$) in the fluorosis group. No significant differences between the fluorosis and the control groups were found for the serum concentrations of total triiodotyronine (TT_3), free triiodotyronine (FT_3), or free thyroxine (FT_4). Significant decreases were present in the levels of TT_3 ($p<0.01$) and FT_4 ($p<0.001$) in the older goats with fluorosis, 3–5-years-old and >5-years-old, compared to the younger animals, aged 1–<3 years.

Keywords: Fluoride; Fluorosis; Goats; Goitre; Thyroid hormones.

INTRODUCTION

Chronic endemic fluorosis may occur in both human beings and animals living in regions with high concentrations of the fluoride ion (F) in the soil and the water. Dental fluorosis is the commonest visible lesion but skeletal fluorosis and soft tissue pathology also occur.[1]

The effect of F on thyroid gland is not fully understood. McLaren[2] speculated that F may accumulate in the thyroid gland and cause structural and functional changes. In contrast, Demole[3] claimed that, F had no specific toxic effect on thyroid gland and did not accumulate in the gland. Bildik[4] reported, in a study performed on sheep, that protein bound iodine (PBI) was lower in sheep with fluorosis compared to normal sheep and speculated that there might be a negative relationship between F and iodine. In another study, Liu et al. reported that if excessive F and excessive iodine were taken together, the functioning of the thyroid would slowly deteriorate. [5]

In the light of above information, that the effects of chronic fluorosis on the thyroid hormones have not been fully clarified, the objective of the present study was to investigate the relationship between the plasma levels of F and the thyroid hormones in goats living in the foothills of Ağrı Dağı (Mount Ararat) and Tendürek Mountain, where chronic fluorosis in goats causes important economic losses.

[a]Department of Internal Diseases, Faculty of Veterinary Medicine, University of Namık Kemal, 59030, Tekirdağ, Turkey; [b]Department of Internal Diseases, Faculty of Veterinary Medicine, University of Erciyes, 38039, Kayseri, Turkey; *For correspondence: Sezai Arslan, Department of Internal Diseases, Faculty of Veterinary Medicine, University of Namık Kemal, 59030, Tekirdağ, Turkey; E-mail: sezaivetgov@yahoo.com

MATERIAL AND METHODS

The animals studied were goats living in villages with high water F concentrations (Ayrancı, Alıntepe, Yılanlı, Gölyüzü, and Çiftlik) located in the foothills of Ağrı Dağı (Mount Ararat) and Tendürek Mountain. The investigations were made on a total 104 goats with signs of chronic dental fluorosis, aged 1–7 yr, from the above locations, together with a control group of 30 healthy goats from Dağdelen village were fluorosis had not been observed. Clinical examinations, body temperatures, heart rates, and ages were recorded for all the animals. The goats with dental fluorosis were also examined for palpable bone exostoses, mandibular defects, rib anomalies, metacarpal and metatarsal defects, nail deformities, lameness, and weakness. Furthermore, the thyroid glands of the animals were examined for the presence of goitre.

The blood samples were taken from jugular veins in plain tubes for serum samples and in tubes containing lithium heparin for plasma. The obtained blood samples were centrifuged at 3000 rpm for 15 minutes to get serum and plasma. The obtained serum and plasma samples were stored at –20ºC until analysis.

The water samples were taken as follows: (i) Çiftlik and Dağdelen villages: from two different drinking fountains; (ii) Alıntepe and Gölyüzü villages: from artesian wells and from creek water because these villages did not have drinking fountains; and (iii) Yılanlı village: from both a drinking fountain and creek water.

The determinations of F in the plasma and water samples were made using a specific fluoride electrode (Orion 96 09 00 BN) with an ionometer (Orion 720 A).[6,7] The serum total triiodotyronine (TT_3), total thyroxine (TT_4), free triiodotyronine (FT_3), and free thyroxine (FT_4) levels were determined using commercial kits (DPC, Immulite) together with an auto analyser (DPC Immulite 2000).

The results were analysed with the SPSS12.0 pocket programme. The comparisons between the goats with fluorosis and the control goats were made with the t test. The comparisons between the goats of different ages, 1–<3-years-old, 3–5-years-old, and >5-years-old, were made with the ANOVA test. The analysis of the relationships between F, the thyroid hormones, and age were made with the Pearson correlation coefficient or Spearman's rank correlation coefficient depending on the distribution of the data. Statistical significance was considered to be present with $p<0.05$.

RESULTS

In the goats showing signs of dental fluorosis, the average body temperature (39.1±0.4ºC), heart rate (76±1.7 beats/min), and respiratory rate (17.2±1.3 breaths/min) were within the physiological limits. Palpation in the neck region gave no evidence of goitre. The colour of the stains on teeth of the goats ranged from yellow to brown. Additional signs of dental fluorosis were also present, depending on the degree of usage, consisting of (i) increased tooth abrasion fractures, (ii) hypoplasia, and (iii) hollows and erosions in the enamel. Also present in the 104 animals with dental fluorosis were: (i) lameness in 8 goats (7.69%), (ii) nail

deformity in 3 goats (2.88%), (iii) palpable bone exostoses in 2 goats (1.92%), and (iv) chewing difficulties and weight loss in 20 goats (19.23%).

The F levels in the water supplies in the villages in the study were: Ayrancı village: 12.5 ppm; Alıntepe village: 12.5–13.7 ppm; Gölyüzü village: 7.2–11.56 ppm; Yılanlı village: 9.5–13.7 ppm; Çiftlik village: 9.56 ppm; and Dağdelen (control village): 0.16–0.53 ppm.

Compared to the healthy goats in the control group, the goats with clinical fluorosis had higher plasma F levels ($p<0.001$) and lower serum TT_4 levels ($p<0.01$). The values for TT_3, FT_3, and FT_4 were not significantly different between the two groups (Table 1).

Table 1. Plasma fluoride ion (F), total triiodothyronine (TT_3), total thyroxine (TT_4), free triiodothyronine (FT_3), and free thyroxine (FT_4) in the goats with fluorosis (n=104) and in the control group (n=30). Values are the mean±standard error of the mean (SEM) and the range

Parameters	Groups	n	Mean±SEM	Range
Fluoride (ppm)	Fluorosis	104	0.24±0.01‡	0.09–0.82
	Control	30	0.11±0.004	0.07–0.18
TT_3 (ng/dL)	Fluorosis	104	86.43±3.05	41.70–212
	Control	30	89.59±4.33	54.80–150
TT_4 (µg/dL)	Fluorosis	104	4.87±0.14†	1.88–9.12
	Control	30	5.79±0.25	3.43–8.50
FT_3 (pg/mL)	Fluorosis	104	2.02±0.06	1.00–4.08
	Control	30	1.87±0.12	1.07–3.80
FT_4 (ng/dL)	Fluorosis	104	0.81±0.02	0.30–1.32
	Control	30	0.85±0.03	0.49–1.30

Comparing the goats with fluorosis with the control group: †$p<0.01$, ‡$p<0.001$.

The plasma F concentrations were significantly increased in the goats with fluorosis compared to the control group ($p<0.001$ and $p<0.05$). The plasma F concentrations tended to be higher in the older animals compared to the younger animals, but not significantly so. Significant decreases were present in the levels of TT_3 ($p<0.01$) and FT_4 ($p<0.001$) in the older goats with fluorosis, 3–5-years-old and >5-years-old, compared to the younger animals, aged 1–<3 years. No significant differences in any of the four parameters with age was seen in the

control groups. The FT_3 was significantly increased in the 1-3-years-old goats with fluorosis compared to the 1-3-years-old control goats ($p<0.05$). The TT_4 and the FT_4 were significantly decreased in the 3–5-years-old goats with fluorosis compared to the 3–5-years-old control goats ($p<0.05$) (Table 2).

Table 2. Plasma fluoride ion (F), total triiodothyronine (TT_3), total thyroxine (TT_4), free triiodothyronine (FT_3), and free thyroxine (FT_4) according to the age groups, 1–<3-years-old, 3–5-years-old, and >5-years-old, in the goats with fluorosis (total n=104) and in the control group (total n=30). Values are the mean±standard error of the mean (SEM) and the range

Parameters	Groups	Total n	Fluorosis and control groups according to age					
			1–<3-years-old		3–5-years-old		>5-years-old	
			n	Mean±SEM	n	Mean±SEM	n	Mean±SEM
Fluoride (ppm)	Fluorosis	104	41	0.22±0.02‡	45	0.25±0.02*	18	0.28±0.04*
	Control	30	15	0.11±0.007	9	0.12±0.006	6	0.12±0.01
TT_3 (ng/dL)	Fluorosis	104	41	99.1±5.7[a]	45	80.78±3.62[b]	18	71.69±5.17[b]
	Control	30	15	97.96±6.13	9	81.79±3.30	6	80.33±11.95
TT_4 (µg/dL)	Fluorosis	104	41	5.24±0.23	45	4.70±0.21*	18	4.44±0.29
	Control	30	15	6.05±0.34	9	5.82±0.47	6	5.09±0.60
FT_3 (pg/mL)	Fluorosis	104	41	2.10±0.10*	45	1.98±0.08	18	1.95±0.15
	Control	30	15	1.69±0.12	9	2.01±0.20	6	2.14±0.39
FT_4 (ng/dL)	Fluorosis	104	41	0.88±0.02[c]	45	0.77±0.02*[d]	18	0.73±0.03[d]
	Control	30	15	0.86±0.06	9	0.88±0.05	6	0.80±0.06

Comparing the goats with fluorosis with the control group: *$p<0.05$, ‡$p<0.001$.

Comparing the goats with fluorosis according to age and comparing the healthy control goats according to age: [ab]the groups with the different lower case letters, a and b, differed significantly, $p<0.05$; [cd]the groups with the different lower case letters, c and d, differed significantly, $p<0.001$; the absence of lower case letters indicates that no significant difference was present.

In the goats with fluorosis, no significant correlation was present between the plasma F levels and the serum thyroid hormone levels ($p>0.05$). However, in these goats, significant negative correlations were present between age and the serum concentrations of TT_3 ($p<0.01$), TT_4 ($p<0.05$), and FT_4 ($p<0.01$) (Table 3).

Table 3. Correlations (Pearson or Spearman correlation coefficients) between the plasma fluoride ion (F), total triiodothyronine (TT_3), total thyroxine (TT_4), free triiodothyronine (FT_3), free thyroxine (FT_4) and age in the goats with fluorosis (total n=104)

Parameters	TT_3	TT_4	FT_3	FT_4	Age
Fluoride	0.094	–0.005	–0.068	–0.016	0.54
TT_3		$0.662^{\dagger}$	$0.402^{\dagger}$	$0.470^{\dagger}$	$-0.339^{\dagger}$
TT_4			$0.373^{\dagger}$	$0.704^{\dagger}$	-0.214^{*}
FT_3				$0.347^{\dagger}$	–0.102
FT_4					$-0.379^{\dagger}$

Statistical significance of the correlation coefficients between the parameters in the columns and the corresponding parameters in the rows: $^{*}p<0.05$, $^{\dagger}p<0.01$.

DISCUSSION

Fluorosis, in both humans and animals, is frequently encountered in the vicinity of the villages of Dogubeyazit and Caldıran, which are located around an old Tendurek Volcano. In studies conducted in these villages, high fluoride ion levels in the water, soil, and feed ingredients have been demonstrated by numerous researchers.[8, 9]

In the present study, the range of the F levels in the drinking water in the villages with the fluorotic goats was 7.2–13.7 ppm, which is very high compared to the guideline for the upper limit of F in drinking water of 1.5 ppm proposed by the WHO in 2011, with the proviso that the volume of water consumed and the intake of F from other sources should be considered when setting national standards.[10] Several investigators have noted that water F levels of the order of 7.2–13.7 ppm can cause fluoride poisoning.[8,11] The water F levels found in the present study were similar to the results obtained in the same area previously by other researchers.[8,9]

Chronic fluoride poisoning causes disturbances in bone and teeth.[1] In a study by Dwivedi et al. on buffalo with fluorosis, the frequencies of various signs were: dental fluorosis 100%, lameness 28.17%, bone exostoses 8.45%, and weight loss 76%.[12] In the present study of 104 goats showing signs of dental fluorosis, the frequencies of other signs were: lameness 7.69%, nail deformity 2.88%, palpable bone exostoses 1.92%, and difficulty in chewing with weight loss 19.23%. No other studies of these signs in goats with fluorosis were found in the literature for comparison. However, when compared to the buffalo with fluorosis,[12] the goats in the present study were less affected by lameness, exostoses, and weight loss. Species differences in the sensitivity of different animal species to F toxicity have been reported, as well as effects related to age and the dietary F intake. [1]

In a study on pigs, where the plasma half-life of F is very short, frequently repeated measurements of plasma samples for the estimation of plasma data were reported to be useful.[13] A relationship between the serum F concentration and the F content of the animal food has also been reported.[14-17]

The range for the normal serum F concentration found by Samal and Naik in tropical sheep in Hirakud, Sambalpur, Odisha, India, was 0.04–0.18 ppm.[15] The mean serum F concentrations found by Suska and Janiak in control Polish Merino and Blackhead Mutton sheep grazing in an area uncontaminated with F, 150 km from Police, Poland, were 0.119 ppm (6.24 μmol/L) and 0.105 ppm (5.50 μmol/L), respectively.[17] The mean F concentration we obtained in the control group of normal goats of 0.11 ppm was found to be close to the findings, 0.04–0.18, 0.119, and 0.105 ppm, of these researchers.

The mean plasma F concentration found by Bennis et al. in goats in the F-contaminated Darmous area of Morocco was reported to be only mildly elevated at 0.31±0.15 ppm (range: 0.10–0.58 ppm), despite the reported F values in the soil, water, and straw of 3232 ppm, 1.2 ppm, and 104 ppm, respectively.[16] Suska and Janiak[17] reported the mean serum F levels in Polish Merino and Blackhead Mutton sheep grazing in a F-contaminated near Police, Poland, were 0.152 ppm (7.98 μmol/L) and 0.134 ppm (7.03 μmol/L), respectively.[17] Oto [6] found the range of plasma F levels between in sheep in an endemic fluorosis was 0.44–0.93 ppm. The mean plasma F level of 0.24 ppm we found in the goats with fluorosis was higher than the values found by Suska and Janiak (0.152 and 0.134 ppm)[17] but lower than those found by Bennis et al. (0.31 ppm) [16] and Oto (0.44–0.93 ppm).[6] These differences can be explained by differences in the species, the ages, the diet, the climate, the sample collection period, and the amount of exposure to F. The F concentration in blood has been reported to increase with advancing age.[15,18,19] In the present study, a similar trend was present in the goats with fluorosis but it did not reach the level of statistical significance ($p>0.05$).

Hoogstratten et al.[14] did not find any macroscopic, histologic, or functional effects of F on the liver and thyroid gland. Jolly et al.[20] examined 26 persons with proven skeletal fluorosis and reported that the morphology of the thyroid gland was normal with no evidence of local and generalized swelling. In the present study, the necks of the goats with fluorosis were examined by both inspection and palpation and the absence of any signs of goitre is consistent with the findings of Hoogstratten et al. [14] and Jolly et al. [20]

Although it is controversial, various researchers differ in their accounts of how fluorosis affects the thyroid hormones.[3,21-24] Some researchers found that F significantly decreased both the T_3 and T_4[25,26] while other researchers reported a significant decrease only in T4 with no significant change in T_3.[27-29] In contrast to these researchers, Zhao et al.[23] reported that, particularly in iodine insufficiency, the levels of the T_3 and T_4 hormones increased with excessive F intake. Our findings, of a significant decrease in the TT_4 but not in the TT_3, FT_3, or FT_4 are consistent with the studies by Zhan et al.,[29] Kapoor et al.,[28] and Yu.[27]

In the present study, significant decreases were present in the levels of TT_3 ($p<0.01$) and FT_4 ($p<0.001$) in the older goats with fluorosis, 3–5-years-old and >5-years-old, compared to the younger animals, aged 1–<3 years. In addition, the TT_3 and TT_4 values tended to decrease with increased age in the control group but not to a level of statistical significance. Similarly, Colavita et al.[30] in a study on healthy goats reported that the T_3 and T_4 decreased with age. Other studies in goats with fluorosis of the changes in the thyroid hormones with increased age were not available for comparison. The decrease in the levels of thyroid hormones with increased age, in both the fluorotic and healthy animals, could be an age-related effect. In addition, the decrease in animals with fluorosis with increased age could be due to an increased F-exposure time. In the present study, the TT_4 and the FT_4 were significantly decreased in the 3–5-years-old goats with fluorosis compared to the 3–5-years-old control goats ($p<0.05$) which is consistent the decline in these hormones with increased age being an effect of increased exposure time. No clear reason was found for the finding, in the present study, that the FT_3 was significantly increased in the 1–3-years-old goats with fluorosis compared to the 1–3-years-old control goats ($p<0.05$).

CONCLUSION

We found that in 104 goats with dental fluorosis in an endemic fluorosis area, mastication difficulties with weight loss occurred in 19.23%, there was a significant decrease in the TT_4, and the values for TT_3, TT_4, and FT_4 decreased with increased age. The present study provides some reference values for future studies on the relationship between the fluoride ion and the thyroid hormones in goats.

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