EFFECT OF CHRONIC FLUORIDE TOXICITY ON GLUCOCORTICOID LEVELS IN PLASMA AND URINE

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SUMMARY: Circulating levels of cortisol were estimated in 14 fluorosis patients and 9 healthy control subjects. The plasma cortisol level was markedly decreased in the patient group. To confirm this, the protocol was repeated in fluoride-treated experimental animals with sex and age matched controls. The fluoride-treated group also demonstrated marked hypocortisolemia, and the decrease was consistent at different time periods irrespective of diurnal variation. The 17-hydroxy-corticosteroid levels in urine was also decreased ($p < .001$), clearly suggesting adrenal hypofunction in chronic fluroide toxicity.

KEY WORDS: Calcium osteopenia; Cortisol; Corticosteriod; Fluorosis; Gluco-corticoids.

Introduction

Glucocorticoids are one of the least studied calcium regulating hormones in chronic fluoride toxicity and fluorosis. At supraphysiologic level, they are known to inhibit bone formation and stimulate bone resorption, resulting in severe bone loss or osteopenia (1-3). Glucocorticoids are also known to inhibit intestinal calcium absorption (4), possibly by inhibiting the synthesis of a calcium binding protein in intestine (5). Enhanced urinary calcium excretion is also very common in subjects having higher circulating levels of endogenously produced corticosteroid (6), or in patients under prolonged glucocorticoid therapy (7).

In order to understand the role of glucocorticoids, if any, in the pathogenesis of bone lesions in chronic fluoride toxicity in man and animals, investigations on glucocorticoid metabolism in experimental animals and cortisol levels in fluorosis patients were planned and conducted.

Materials and Methods

Osteofluorosis Patients and Control Group: Patients (n = 14) were selected from the outpatient clincis and in-patients of the All India Institute of Medical Sciences Hospital, New Delhi. In each case, a detailed clinical and radiological examination was conducted and the diagnosis was reconfirmed. The control group consisted of nine healthy individuals of comparable age, sex and weight.

Experimental Animals: Sixteen male rabbits were randomly distributed in two grups of 8 each and kep under identical laboratory conditions with 16:8 hr dark and light cycles. One group of animals (n = 8) was orally adminsitered

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NaF solution at the dose of 10 mg NaF/kg body weight daily for a period of 24 months. The other group (n = 8) served as controls.

Sampling of Body Fluids: In view of the fact that there is a diurnal variation in the plasma concentration of corticosteriods, blood samples were collected from the patients and their matched controls at identical time intervals. The same protocol was maintained for the experimental animals. After the blood was collected in a heparanized tube the plasma was separated by centrifugation, using a refrigerated centrifuge, and stored at -20°C until use.

Twenty-four hour urine was collected for 3 consecutive days; hormone assay was carried out on samples collected on each day; the mean of the 3 day assay value was reported as the final result.

Plasma Cortisol Level in Human Subjects: total cortisol levels in plasma were estimated by Radioimmunossays according to WHO Assay Method Manual (8).

Corticosteroid Levels in Plasma and Urine in Experimental Animals: Because of the controversy as to whether cortisol and corticosterone is the major corticosteroid in rabbit, both cortisol and corticosterone in the plasma of the rabbit were investigated. Competitive protein binding radioassay procedure was adopted (9). Urinary corticosteroid was also assayed by the same method.

17-Hydroxycorticosteroid (17 OHCS) Levels in Urine: Urinary 17 OHCS in experimental animals were established according to the method of Silber and Porter (10).

Statistical Method: The significance of the differences between the mean values was established by the Student's t-test.

Results

Cortisol Levels in Patient and Control Groups: The 14 Osteofluorosis patients were subdivided into three groups according to their blood collection time and each group had a matched control group with same blood sampling time. All three groups of fluorosis patients showed hypocortisolemia, and the decrease was statically significant compared to control values (Table 1).

Table 1

Plasma Cortisol (Total) Levels in Fluorosis Patients

	Age (in years) (Mean ±S.D.)	Total Cortisol (µg/100mL). (Mean ±S.D.)		
		10:30#	11:30#	12:30#
Control Group (n = 9)	37.22 ±9.03	14.01 ±0.84 (n = 3)	11.66 ±0.77 (n = 3)	9.68 ±0.63 (n = 3)
Patient Group (n = 14)	43.28 ±11.20	8.68 ±1.62* (n = 5)	7.45 ±1.12** (n = 5)	5.73 ±0.98* (n = 4)

Blood Sample Collecting Times; * $p < 0.001$; ** $p < 0.005$

Corticosteroid Levels in Plasma and Urine in Experimental Animals: Both cortisol and corticosterone levels in plasma were estimated to find out the main corticosteroid in the experimental rabbits. The 8 A.M. blood sample clearly demonstrated cortisol as the main corticosteroid in the experimental animals (Table 2). Plasma cortisol, as well as corticosterone, showed a decrease in the fluoride-treated group compared to the values of the control animals (Table 2).

Plasma cortisol levels at three different time periods namely, 7:00 am, 12:30 pm and 5:00 pm were also investigated to find out whether the hypocortisolemia observed in fluoride-treated animals is maintained at different time periods of diurnal periodicity.

As in Osteoflurosis patents, the fluoride-treated animals also demonstrated hypocortisolemia at all three different time periods of circadian periodicity and the difference was statically significant (Table 3).

The urinary free cortisol, however, did not show any deviation from control values (Table 4).

Table 2

Plasma Corticosteriod Levels in Experimental Animals (8:00 am)

Animal Group	Body Weight (in grams) Mean ± S.D.	Duration of Fluoride Treatment# (in months)	Cortisol (µg/100 mL) Mean ±S.D.	Corticosterone (µg/100 mL) Mean ±S.D.
Control (n = 8)	1607.14 ±128.50	Nil	7.21 ±1.56	2.29 ±0.38
Treated (n = 8)	1557.14 ±123.92	24	4.35 ±0.63*	1.39 ±0.41*

#Dose: 10 mg NaF/kg body wt./day; * $p < 0.001$

Table 3

Plasma Cortisol (Total) Levels in Experimental Animals

Animal Group	Plasma Cortisol Level (µg/100 mL) (Mean ±S.D.)		
	7:00#	12:30 pm#	5:00 pm#
Control (n = 8)	7.96 ±1.18	4.10 ±0.65	2.18 ±0.36
Treated (n = 8)	5.01 ±0.46[a]	2.88 ±0.45[a]	1.73 ±0.35[b]

@ Fluoride-treated for 24 months; # Blood Sampling Time;
[a] $p < 0.001$; [b] $p < 0.025$

Table 4

Urinary Levels of Cortisol and 17-OHCS in Experimental Animals

Animal Group	Urinary Excretion	
	Cortisol (µg/24 hr) (Mean ± S.D.)	17 OHCS (µg/24 hr) (Mean ±S.D.)
Control (n = 8)	4.37 ±0.83	237.50 ±31.05
Treated (n = 8)	4.23 ±1.01	168.12 ±31.61*

* $p < 0.001$

Urinary 17 OHCS Levels in Experimental Animals: Unlike the free cortisol, the 17 OHCS levels in urine was markedly decreased in the fluoride-treated group ($p < 0.001$). This decrease in 17 OHCS is consistent with the decreased corticosteroid levels in plasma, thus suggesting hypofunction of adrenal gland.

Discussion

In chronic fluoride toxicity and skeletal fluorosis the pathogenesis of bone tissue is not clearly understood. Disturbed metabolism of some of the calcium regulating hormones has been considered as one of the main factors responsible for bone lesions in chronic fluoride toxicity and fluorosis. Hypersecretion of parathyroid hormone and osseous changes consistent with hyperparathyroidism are established facts in chronic fluoride toxicity (11). Calcitonin is also raised in chronic fluoride toxicity (12), although some contradictory results have been reported (13). Srivastava et al. (14) reported subnormal vitamin D_3 levels in two of the six fluorosis patients investigated, whereas some reports suggest no alteration in vitamin D_3 levels (15).

Although glucocorticoids are very important calcium regulating hormones, they have received scant attention in fluoride toxicity research. Rao and Susheela (16) reported decreased activity of delta 5-3-β hydroxysteriod dehydrogenase, thereby suggesting impaired steriod production in chronic fluoride toxicity (17). Guansheng Li found reduced levels of sterum corticosterone in fluoride-treated rats (18).

The observation on hypocortisolemia in skeletal fluorosis patients reported in this communication is perhaps the only one of its kind. The above finding was further confirmed by the results of the experimental animals. In the animal study cortisol level was decreased in the fluoride-treated group irrespective of circadian variation, thus suggesting adrenal insufficiency in chronic fluoride toxicity. Urinary 17 OHCS was also decreased in the fluoride-treated group. As 17 OHCS levels in urine is a specific assessment of overall cortisol production, the decreased levels of 17 OHCS likewise suggest hypofunction of the adrenal gland. This study, owever, is inadequate to explain the normal excretory levels of free cortisol and needs further investigation.

It is a well known fact that bone formation is enhanced in chronic fluoride toxicity (19), whereas glucocorticoids at supraphysiologic levels are

a very potent inhibitor of bone formation (1). Therefore, fluoride by inhibiting cortisol production, indirectly may be stimulating different factors responsible for bone formation. The increased intestinal calcium absorption and hypocalciuria observed in chronic fluoride toxicity (20)a could aslo be due to decreased levels of cortisol, since glucocorticoids at supraphysiologic levels have an antagonistic effect.

Thus, in conclusion, it can be stated that fluoride, by inhibiting cortisol production, i probably disturbing the delicate balance between the calcium regulating hormone and bone metabolism, thereby creating an environment/ millieu which may be one of the causative factors for the skeletal derangements in fluorosis.

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