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Research Article

SYNDROME OF LOW TRIIODOTHYRONINE IN CHRONIC FLUOROSIS

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ABSTRACT
The present study examined the thyroid function and low T3 state in a group of patients exposed to different concentration of fluoride in drinking water. 139 subjects from severe endemic fluorosis areas and 140 subjects as control group were randomly selected. The functional activity of thyroid gland was measured and the findings indicate that the level of TSH and rT3 was significantly ($P<0.001$) elevated and of T3 was declined in the fluorotic group. The T4 concentration showed significant ($P<0.05$) elevation in A-III and A-IV groups, however the elevation in A-I and A-II was not statistically significant. The level of fluoride (F) in serum and urine was significantly ($P<0.001$) higher in fluorotic study groups as compared to control group. Pearson's bivariate coefficient of correlation revealed a positive relationship between water F vs. TSH ($r= 0.98$), water F vs. rT3 ($r= 0.77$) in different study groups. An inverse correlation existed between serum F vs. T3 ($= -0.82$) and serum F vs. T4 ($r= -0.88$). The study demonstrate that abnormalities in thyroid function characterized by a low level of T3, high rT3 and a slight increase of the TSH with normal to low T4 indicating low T3 syndrome in cases of chronic fluoride intoxication. It is also evidenced that fluoride in excess may be inducing disease normally attributed to iodine deficiency. The normal or optimal levels of iodine in the urine and the low level of T3 with higher level of rT3 can serve as a diagnostic sign of chronic fluoride exposure.

Key Words: *Fluorosis, TSH, T3, T4, rT3*

INTRODUCTION
Thyroid hormones are fundamental for the development, growth, reproduction and metabolism (Zoeller *et al.*, 2000). Thyroxine (T4) is the main secretion of thyroid gland, but the receptor-active thyroid hormone is 3, 5, 3'-triiodothyyronine (T3). Serum levels of thyroid hormones, including T3, T4 and Thyroid stimulating hormone (TSH) are commonly used a reliable indicator of the thyroid function in humans and experimental animals. Changes in the serum concentration of these hormones can reflect disturbances in their glandular synthesis as well as disorders in their extra thyroidal peripheral metabolism (Kelly, 2000).
Several conditions for abnormal plasma levels of thyroid hormones have been reported in patients with a variety of diseases, the important of which is low T3 syndrome. It is not a true syndrome but rather reflects alterations in thyroid function test in a variety of clinical situations that commonly includes a low serum T3, normal or low T4 and a high reverse T3 (rT3) (Alder and Wartofsky, 2007). The T3/rT3 ratio has been used as the most useful biomarker for tissue hypothyroidism and diminished cellular functioning (van den *et al.*, 2005). The low T3 state has been described in surgery (Cherem *et al.*, 1992), cardiopulmonary bypass (Holland *et al.*, 1991), bone marrow transplantation (Vexiau *et al.*, 1993) and respiratory syndrome (Scoscia *et al.*, 2004).
The presence of synthetic chemicals in the environment is currently a major problem for both human and animal health. Compounds used in industry and agriculture most of the time end up in the ecosystems. Due to their chemical properties such as lipophilicity, chemical stability and miscibility with organic compounds, some of them are still present long after their use has been forbidden or finished Coimba *et al.*, 2005). Environmental chemicals that act on endocrine systems interfering and altering their function are called endocrine disruptors (Colborn, 2010). The effects of several environmental contaminants on the thyroid axis remains to be investigated, to clearly understand how these compounds interfere with thyroid function.

Fluoride is a microelement for human health but has been listed among the most significant endotoxins that appear in natural environment as after-effects of industrial activity of humans. Fluoride ions, after absorption to the blood from gastro intestinal tract or the lungs, easily penetrate to the cells through membranes (Birkner *et al.*, 2008) and affects different mechanisms (Wu *et al.*, 2008). It is known to accumulate not only in bones and teeth but also in soft tissues (Cinar and Selcuk, 2005). Fluoride can affect the hormone secretion of the thyroid (Hu *et al.*, 2007; wang *et al.*, 2009; Shashi and Singla, 2009). Excessive long term intake of fluoride is a significant risk factor for the development of thyroid dysfunction (Yaming *et al.*, 2005). To date, there are few data on the thyroid function in patients with chronic fluoride toxicity, and there are no data available on low T3 syndrome in fluorotoxicity except, in workers of chronic occupational exposure (Mikhailets *et al.*, 1996) and in children with high fluoride in drinking water (Susheela *et al.*, 2005).

The present study examined the thyroid function and low T3 state in a group of patients exposed to different concentration of fluoride in drinking water. This study was approved by the institution Human Ethics Committee of our institution.

MATERIALS AND METHODS

Study Design

A total of 279 adults aged 22-47 (mean age 34.80 ± 9.40) of both the sexes were randomly selected, out of which, 139 from fluoride endemic areas of Bathinda district, Punjab, India and 140 from non fluorotic areas as control group.

Blood Sampling and Processing

Fasting venous blood samples were collected from the selected patients and controls in non heparinized vacutainers and left for 20 minutes to allow clotting . Clear sera were obtained by centrifugation at 2000 rpm for 15 minutes and stored at 20°C for further biochemical analysis.

Biochemical Assays

Thyroid function was evaluated by measuring serum levels of TSH, T3, T4 and reverse T3 (rT3) with a direct enzyme immune assay (Biocheck, Inc. California and BioMontecelio, Italy). The protein levels in serum of control and fluorotic patients were determined by method of Lowry *et al.*, (1951), albumins was estimated by modified Bromocresol Green method (McPherson and Everard, 1972).

The estimation of fluoride in water and serum was done by using Orion ion selective electrode (EA940, Boston, MA, USA). On the basis of water fluoride concentration, the study areas were divided into five subgroups viz: control (0.65-1.00 mg/l), A-I (1.01-4.00 mg/L), A-II (4.01-8.00 mg/L), A-III (8.01-12.00 mg/L) and A-IV (12.01-16.00 mg/L).

Data Analysis

All the data was expressed as mean ± standard deviations (S.D.). One way analysis of variance (ANOVA) with post-hoc analysis was used to compare the variables in different groups. Association between variables was assessed by Pearson's bivariate cofficient of correlation. Two sided P values of <0.05 were considered statistically significant. The statistical program used was SPSS for windows version 16.0 (Statistical Package for Social Sciences Inc., Chicago, Illinois, USA).

RESULTS

Thyroid Hormone Levels

One way ANOVA analysis revealed significant ($F_{4,278}$= 22.794, $P<0.001$) decline in the mean level of serum TSH in patients of study groups A-I to A-III (fluoride exposure 1.01-12.00 mg/L) and elevation in study group A-IV where the fluoride concentration was highest (12.01-16.00 mg/L) (Fig. 1) Tukey's LSD multiple comparison test further revealed that the level of TSH altered significantly (q= 1.59 to – 1.08, 95% CI = -2.73 to 4.91, $P<0.05$-0.001) among fluorotic patients of all study groups as well as compared to control group.

The mean serum level of T3 and T4 showed a significant (P<0.001) decrease in patients of fluorosis of all study groups (Fig. 1).

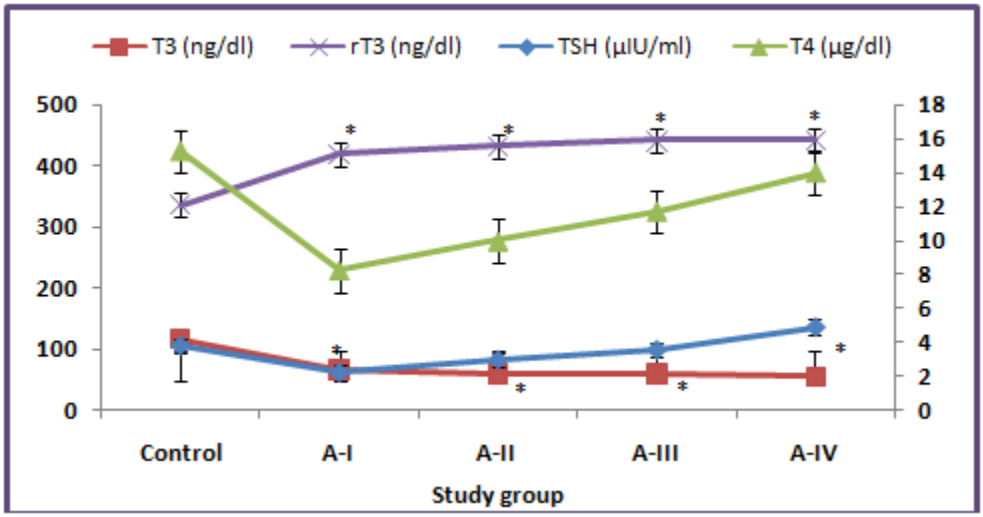

Figure 1: Serum levels of thyroid hormones in fluorotic patients suffering from Low T3 syndrome in different study groups

One way ANOVA with post hoc analysis demonstrated a highly significant ($F_{4, 278}$ =825.067, P<0.0001) variance in the level of T3 in control and fluorotic patients with concomitant increase in water fluoride level (A-I to AIV). Tukey's LSD multiple comparison test revealed that the level of T3 decreased significantly (q=50.13 to 60.05, 95% CI= 29.23 to 89.06, P<0.001) in all fluorotic patients. Bonferroni multiple comparison analysis illustrated that the mean level of serum T4 declined significantly (t= 5.44 to 2.36, 95% CI= -1.33 to 11.75, P<0.05 -0.001) in fluorotic patients of all study groups as well as compared with control. However, differences between study groups A-I and A-II were not statistically significant (P= 0.79).

Patients affected with fluorosis exhibited highly significant ($F_{4, 278}$ = 989.363, P<0.001) increase in mean serum level of rT3 (Fig. 1). The maximum effect on the concentration of rT3 was seen in study group A-IV, exposed to 12.01-16.00 mg/L of fluoride. Tukey's LSD multiple comparison test revealed that the level of rT3 increased significantly (q= 133.77 to 165.26, 95% CI = -156.75 to -133.50, P<0.05 -0.001) in fluorotic patients from all the study groups as well as compared with control.

Subject Characteristics by Rt3 Tertile

The study subjects were divided into three tertiles based on serum concentration of rT3. The lowest tertile (within normal reference range) has concentration of rT3 (<250 ng/dl, n=140); middle tertile has concentration of rT3 (250-350 ng/dl, n= 72); and highest tertile has concentration of rT3 (>350 ng/dl, n= 67) as shown in Table 1. The concentration of rT3 was significantly (P<0.05) increased in all tertiles with advancing age. The one way ANOVA followed by Post hoc Tukey's LSD multiple comparison showed significant ($F_{4, 278}$ =103.625, q=1.35 to -2.54, 95% CI= -10.46 to 11.28, P<0.05-0.001) alterations in the level of TSH in all the tertiles.

The level of T3 was significantly ($F_{4, 278}$= 1957.35, p<0.0001) declined in middle as well as highest tertiles as compared to lowest tertile. Tukey's multiple comparison test also exhibited a significant (q= 55.28 to 4.77 95% CI = -322.55 to 447.01, P<0.001) decrease in T3 concentration in both middle and highest tertiles as well as compared with lowest tertile.

A highly significant (P<0.001) decrease was recorded in mean serum level of T4 in patients affected with low T3 syndrome in middle tertile. The patients of highest tertile had T4 levels within the lower limit of reference range. One way ANOVA with Post hoc Tukey's LSD multiple comparison test described the

significant ($F_{4, 278}$= 125.070, q=4.47 to 2.36, 95% CI= -20.16 to 27.59, P<0.05) variance in the serum level of T4 in all these tertile groups with increase in the serum rT3 concentration.
The ratio of TSH/T3 ($F_{4, 278}$=46.81) and TSH/T4 ($F_{4, 278}$=63.66) showed highly significant (P<0.001) increase in middle and highest tertile in comparison to the lowest tertile. Tukey's LSD multiple comparison test revealed that increase in the ratio of TSH/T3 and TSH/T4 in both middle as well as highest tertile groups, positively differed (q= -0.217 to -0.16, 95% CI= -2.281n to 2.255; q= -1.88 to -0.32, 95% CI= -15.29 to 15.37, P<0.05-0.001 respectively) among the groups as well as compared with lowest tertile.
One way ANOVA with post hoc analysis described a extremely significant ($F_{4, 278}$ = 198.011, q=1.44 to 2.54, 95% CI= -19.59 to 27.16, P<0.001) decrease in ratio of T3/T4 in middle and highest tertile with increase in the serum rT3 concentartion (Table 1).

Table 1: Various characteristics of fluorotic patients of low T3 syndrome by rT3 tertile

Variables	Lowest tertile rT3<250 n=140	Middle tertile rT3 250-350 n=72	Highest tertile rT3>350 n=67
TSH (µIU/ml)	3.82±1.03	2.47±0.85*	5.02±1.25*
T3 (ng/dl)	115.98±10.28	60.70±4.11*	55.93±2.67*
T4 (µg/dl)	11.45±2.45	6.98±1.17	9.09±1.54
TSH/T3	0.033±0.001	0.25±0.002*	0.41±0.005*
TSH/T4	0.33±0.01	2.21±0.85*	2.53±0.95*
T3/T4	10.13±1.65	8.69±1.02*	6.15±0.86*

**P<0.001*

Proteins
Fluorotic patients affected with low T3 syndrome exhibited highly significant (P<0.001) decrease in the mean serum levels of total proteins (TP), albumins (AL) and globulins (GL). The maximum effect of fluoride was observed in study group A-IV (Fig. 2).

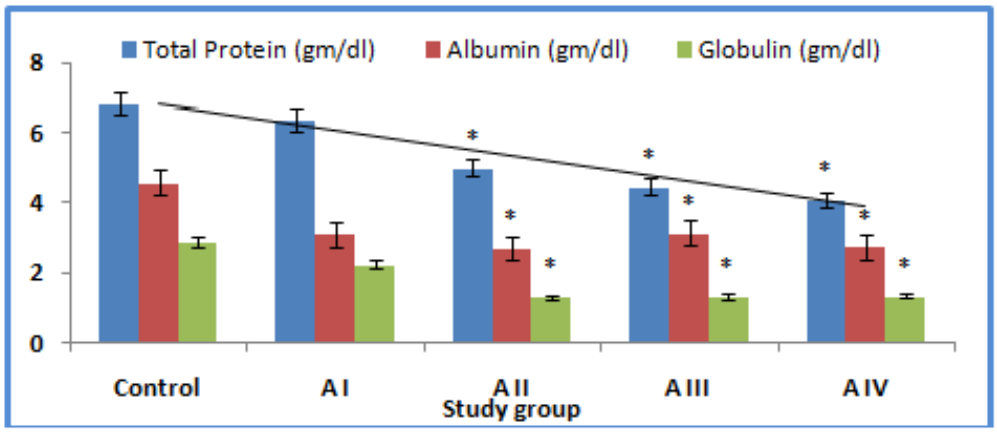

Figure 2: Mean serum levels of proteins in control and fluorotic subjects of different study group

The fluorotic patients with elevated levels of rT3 and low T3, depicted a highly significant ($F_{TP\ 4,278}$ = 251.325, $F_{AL\ 4, 278}$= 91.19, $F_{GL4, 278}$ = 227.385, p<0.001) variance to the null hypothesis with increase in water fluoride concentration. Bonferroni multiple comparison test illustrated that the mean serum levels of proteins (TP, AL, GL) decreased significantly (t_{TP}= 0.47 to 2.73, 95% CI= -7.53 to 9.97, P<0.001);

(t_{AL}= 1.49 to 1.85, 95% CI= -3.33 to 6.22, P<0.001); (t_{GL}= 0.64 to 1.54 95% CI= -3.99 to 5.773, P<0.001) in fluorotic patients of low T3 state of all the endemic fluorosis areas as well as compared with control.

Serum Fluoride

The concentration of serum fluoride in fluorotic patients affected with low T3 syndrome exhibited a stepwise increase in all the study groups. One way ANOVA with post hoc Tukey's LSD multiple comparison test showed significant ($F_{4, 278}$= 11.431, q= 0.30 to 0.56, 955 CI= -1.66 to 0.67, P<0.001) increase in serum fluoride concentration of all the study groups. There was a highly significant (P<0.001) positive relationship between fluoride in water and serum (r= 0.92). Furthermore, the fluoride levels in serum increased gradually as drinking water fluoride level increased.

Correlation Analysis

We compared levels of water fluoride, serum fluoride, thyroid function hormones and rT3 among all patients from different study groups. A significant (P<0.01) positive relationship was observed between the concentration of fluoride in drinking water and serum TSH (r= 0.98, fig 3) and rT3 (r= 0.77 P<0.04, Fig. 4). Linear regression analysis indicated that drinking water fluoride is a strong predictor of alterations in serum fluoride (y=0.165x-0.119, R^2= 0.991); T3 (y= -12.5x =109.4, R^2= 0.63); T4 (y= -2.083x+11.05, R^2= 0.733); TSH (y= 8.084x-5.962, R^2= 0.96); rT3 (y= 35.60x+235.8, R^2= 0.661).

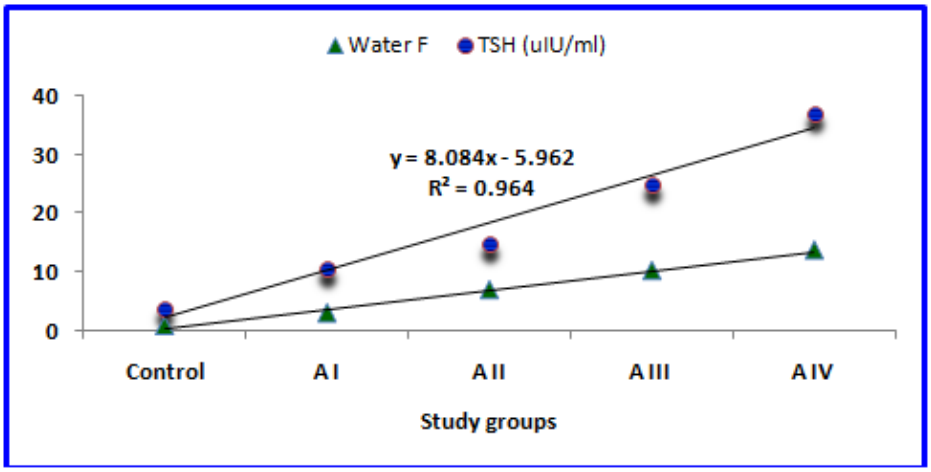

Figure 3: Correlation between water F and TSH in different study groups

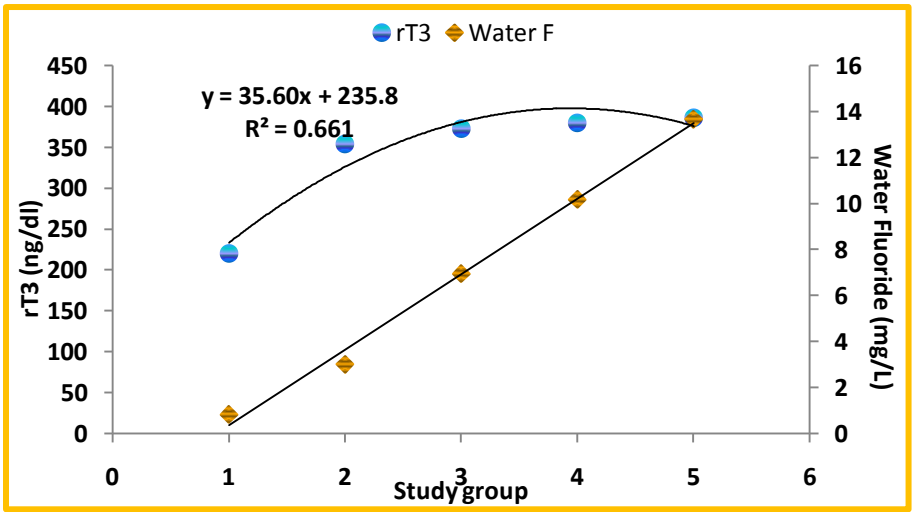

Figure 4: Correlation between water F and rT3 in different study groups

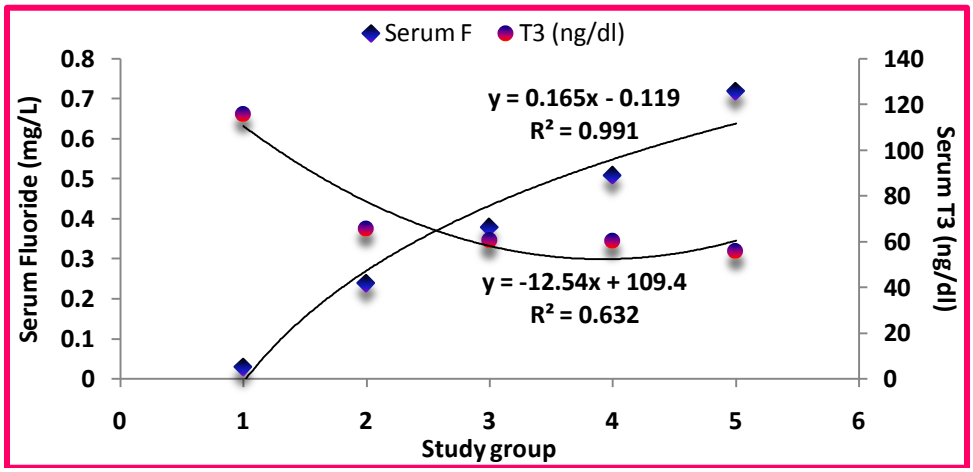

Figure 5: Correlation between serum F and T3 in different study groups

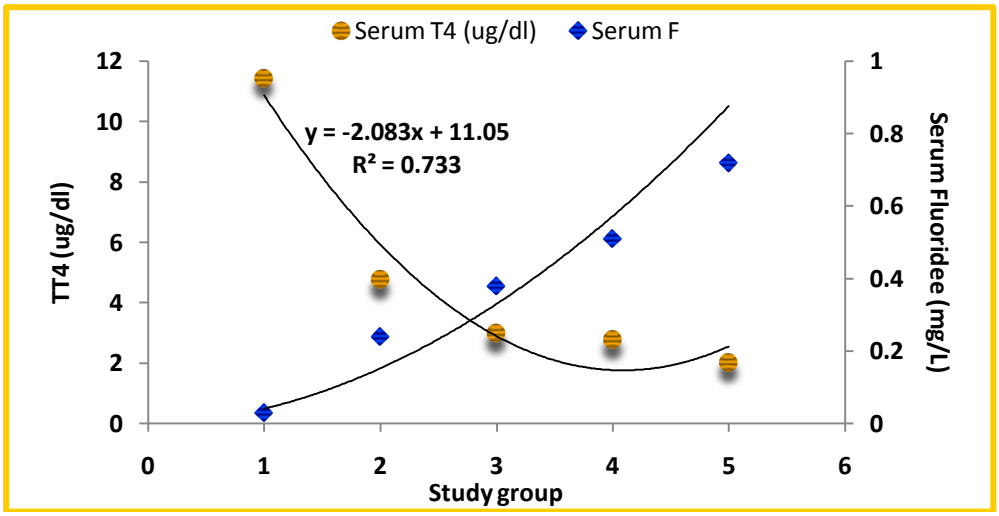

Figure 6: Correlation between serum F and T4 in different study groups

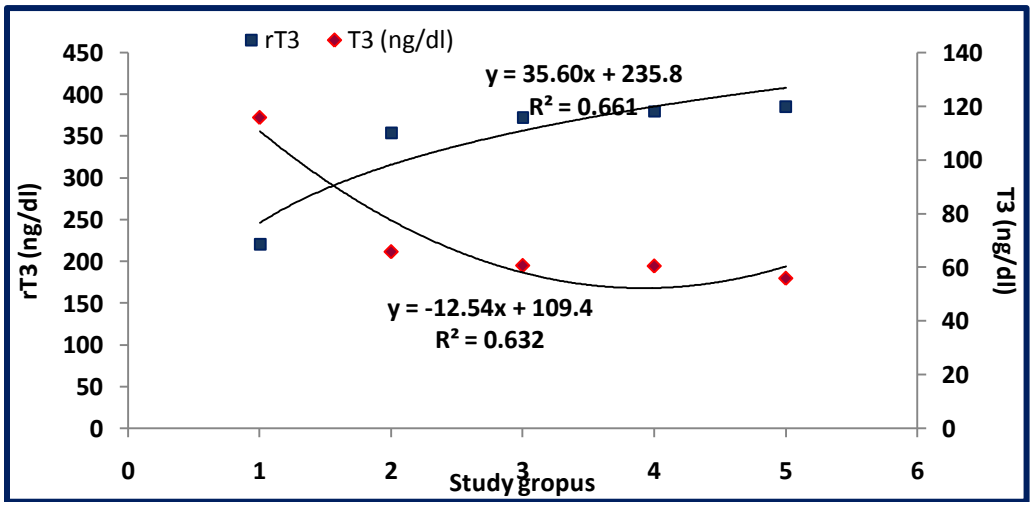

Figure 7: Partial correlation between T3 and rT3 in different study groups

Simple linear correlation and regression analysis showed that lower levels of T3 and T4 were strongly associated with the increase in the level of serum fluoride in different study groups. A significant ($P<0.03$) inverse relationship was found between level of fluoride in serum and T3 ($r= -0.82$, fig 5), T4 ($r= -0.88$, Fig. 6). Linear regression analysis indicated the extremely significant ($P<0.001$) negative causal relationship between serum T3 and rT3, where negative partial correlation was existed in both ($P<0.001$). The regression equation for serum T3 and rT3 was $y=35.60x+235.8$, $R^2= 0.661$. This R square indicated that the serum T3 was a strong predictor of alteration in rT3 in different study areas with increase in water fluoride concentration (Fig. 7).

DISCUSSION

In the present study it was observed that the water fluoride concentration was significantly higher in study groups as compared to control group. It was also clear from the observation that, fluoride in drinking water is the main source of the fluoride intake as reported by the other workers (Xiang *et al.,* 2004; Xiang *et al.,* 2005; Xiang *et al.,* 2009). Therefore it was quite easy to explore the exact relationship between the drinking water fluoride and the serum concentration of TSH and thyroid hormones (T3 and T4).

The present study demonstrates significant relationship between the drinking water fluoride, serum fluoride and the serum concentration of TSH, $T3$, T4 and rT_3 of four study areas and control. rT_3 is an isomer of T_3 with no demonstrated biological activity. It results from the transformation of T_4 through inner ring deiodination by Type I and Type III deiodinases in peripheral tissue. In contrast, outer ring deiodination by Type I and Type II deiodinases leades to activation of T4 into T3 (Moreno *et al.,* 1994; Bianco *et al.,* 2002). Increased rT_3 is considered as a part of euthyroid sick syndrome. This entity is characterized by a constant decrease in serum T_3 and variable abnormalities of other thyroid hormone levels (McIver and Gorman *et al.,* 1997). As in the present study, it was noted that the level of serum rT_3 was significantly ($P<0.001$) higher, T_3 lower and of T4 normal to lower in fluorotic patients as compared to control group. This increase in rT_3 with increase in fluoride concentration may be due to decreased metabolic clearance of rT_3 by 5-deiodinase or increased rT_3 production from 5-deiodenation of T_4 to rT_3. The study showed a strong association between rT3 and fluoride concentration in water and in body fluids. This high rT3 syndrome might precede an overt low T3 syndrome or might be an equivalent of it in the fluorotic patients. This rT3 could deserve to be assessed in fluorotic subjects with normal TSH.

It was suggested that rT_3 could be a reliable hormonal parameter to assess nutritional status (Goichot *et al.,* 1994). The elevated rT_3 may reflect more than simply the nutritional status and also a poor overall health status in endemic fluorotic areas. As in the present study, the level of total proteins, globulins and albumins were significantly ($P<0.001$) lower than the control group indicative of poor health status of the study group.

D_3 deiodinase converts T_4 into the metabolic reverse T_3 and further, T_3 into 3-3'-T_2. D_3 has only an inner ring deiodinase (IRD) activity and an inactivating enzyme. Fluoride is known to interfere with the activity of the deiodinases (Susheela *et al.,* 2005). Lin *et al.,* (1991) found increased reverse T_3 levels, formed by excessive D_3 activity in children. The balance of active T_3 and inactive rT_3 in the serum reflects thyroid hormone economy.

A decrease in T_3 concentration which increases with increase of the fluoride exposure and a more advanced fluorosis stage is quite corresponds to hypothyroidism. The low T_3 level syndrome is due to the effect of fluoride on the peripheral conversion of T_4 toT_3 at the cell target level, although this effect may be indirect or may result from the disruption of the functional activity of the other endocrine gland. In the present study, low level T3 syndrome and increase in TSH was observed in cases of chronic fluoride intoxication. The highest frequency of low level T3 syndrome has been reported in industrial worker affected with toxic liver damage (Scoscia, 2004) starvation or undernourishment (Hennman *et al.,* 1988)

The highest frequency of occurrence of the low T_3 concentration syndrome was seen in study group A-IV with highest level of fluoride in drinking water and may be associated with liver damage, which is frequently observed in fluorosis. Liver must be playing a crucial role in causing low level T_3 syndrome

because of peripheral deiodenation of T4 occuring in liver parenchyma (Mikhailets *et al.*, 1996). The low level T_3 syndrome reported in many acute and chronic pathological states and metabolic conditions similar to starvation or undernourishment may result in lower metabolism which is aimed at energy conservation.

It may be concluded from the present study that abnormalities in thyroid function were characterized by a decrease iodine absorption function of the thyroid, a low level T_3 syndrome and a slight increase of the TSH level in the different study groups. It is also evidenced that fluoride in excess may be inducing disease normally attributed to iodine deficiency. The normal or optimal levels of iodine in the urine and the low level of T_3 with higher level of rT_3 can serve as a diagnostic sign of chronic fluoride exposure.

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