Clinical and Biochemical Profile of Deiodinase Enzymes and Thyroid Function Hormones in Patients of Fluorosis

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Abstract: The thyroid involvement is very significant feature of fluorosis due to fluoride accumulation in soft tissues. The participation of long term consumption of fluoride in drinking water on thyroid hormone status and deiodinating enzymes in fluorotic patients selected randomly from various parts of fluoride endemic areas, was elucidated. Type I (D1) activity was depressed significantly (P<0.001) in all fluorotic study groups in comparison to control, while type-II (D2) activity was lowered significantly (P<0.05) only in study group A-III (8.01-12.00 mg/L) and A-IV (12.01-16.00 mg/L). Serum TSH level was significantly (P<0.001) increased in fluoride exposed groups in comparison to control group whereas the level of rT3 showed a stepwise elevation with increase in water fluoride concentration. Decreased triiodothyronin (T3), thyroxine (T4), free T3 (FT3), free T4 (FT4) levels in serum were noted in all the fluorotic study groups (P<0.01) in comparison to the control. Pearson's bivariate correlation analysis revealed significant (P<0.05-0.001) negative relationship between serum F vs D1 (r= -0.93); serum F vs D2 (r= -0.95); serum F vs T3 (r= -0.84) and serum F vs T4 (r = -0.87). There was direct correlation between serum F and rT3 (r = 0.84, P<0.004). Partial correlation analysis exhibited positive relationship between T3 and D1 (r= 0.96) and negative relationship between rT3 and D1. The regression coefficient of activity of D1 and serum F in all fluorotic paients revealed that water F being the strong predictor of involvement in depletion of D1 activity and increased levels of serum F. The lower activity of both D1 and D2 contribute to decrease in conversion of T4 toT3 and increased conversion of T4 to rT3. The depressed activity of D1 results in reduced conversion of rT3 into T2 and thereby allowing rT3 to accumulate in the body, resulting in hypothyroidism in fluoride intoxication.

Key words: Deiodinase enzyme, Fluoride, Fluorosis, Thyroid hormone

INTRODUCTION

The follicular cells of the thyroid gland produce thyroid hormones thyroxine (T4) and triiodpthyronin (T3) which modulate a variety of physiological processes (Larsen *et al*.2002). The main secretion product of the thyroid gland is thyroxine, but the receptor active thyroid hormone is 3,5,3'-triiodothyronine. The enzymes responsible for thyroid hormones deiodination are called iodothyronine-deiodinases (St.German and Galton, 1997). The enzyme 5'-iodothyronine deiodinase is responsible for converting T4 to T3, the bioactive hormone. Based on several functional criteria, tissue distribution and on different protein sequence, 5,-deiodinases are classified into two isoenzymes: type1(D1) and type 2(D2). D1 is predominantly found in thyroid, liver and kidney and is responsible for generating most of the circulating T3. D2 is expressed in brain and pituitary where it catalyse the local T4 to T3 conversion (Bianco *et al*.2002). However, it has been shown that the contribution of D2 to serum T3 may also be significant (Nguyen *et al*.1998; Sabatino *et al*.2000).

The activation of T3 is carried out in peripheral tissues by deiodination of the outer ring (ORD) of T4. The deiodination of the inner ring (IRD) results in the production of the inactive metabolite3,3,'5-T3 or reverse T3 (rT3) (Visser,1996). IRD also inactivates T3 by converting it to 3,3'diodothyronine (T2). Deiodination controls plasma and tissue thyroid hormone level and the biological activity of these hormones. A broad range of chemicals, with structural similarity to thyroid hormones, have been shown to bind to thyroid receptors with both against and antagonist effects on thyroid hormone signaling (Patrick, 2009). Chemicals that affect thyroid metabolism, either through the hypothalamic-pituitary axis or directly via nuclear receptor are termed thyroid disruptors.(Diamanti *et al*.2009).

Fluorine is the most electronegative element, distributed ubiquitously as fluorides in nature. Water is the major source of fluoride intake by humans. Excessive exposure to fluoride causes toxicity and chronic metabolic disorders in animals and humans. It can rapidly cross the cell membrane and is distributed in the hard and soft tissues. In endemic fluorosis areas or in the cases when fluoride treatment is used, the secretion of T4 and T3 from thyroid could be influenced under adequate intake of iodine (Zhao *et al*., 1998).

Aim of the study:

The aim of this study is to investigate the effects of high fluoride ingestion in drinking water on biosynthesis of thyroid hormone and on deiodinase activity in fluorotic patients.

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MATERIALS AND METHODS

50 adults (40 from fluoride endemic areas, and 10 from non fluorotic areas) aged 25-35 (mean age 30.40 ± 2.40) of both the sexes were randomly selected from high fluoride endemic area of district Bathinda, Punjab, India. On the basis of fluoride concentration, the areas were divided into five groups viz; control (0.76-1.00 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)., kept as control. Fasting venous blood samples (3ml) 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 4 C in the refrigerator for further biochemical analysis.

Deiodinase Activity Measurement:
The activity of deiodinases was measured by the method of Lisboa *et al*. (2001) with slight modifications.

Serum thyrotropin (TSH), T3 and T4 quanification:
Serum levels of TSH, T3, T4 and reverse T3 (rT3) were determined with a direct enzyme immune assay using kit methods (Bio check, Inc. California and Bio,Montecelio, Italy).

Statistical Analysis:
Values are given as Means±SD. Statistical significance was determined by one-way ANOVA followed by Tukey's Kramer multiple comparison test. Association between variables was assessed by Pearson's bivariate cofficient of correlation. The level of significance was set at $P<0.05$. The statistical program used was SPSS for windows version 16.0 (statistical package for the social sciences inc., Chicago, Illinois, USA).

Results:
Deiodinase Activities:
The activity of deiodinase type 1 (D1) was significantly (F= 382.973, P<0.01) decreased in patients of all fluorotic study groups (Figure 1). The maximum D1 activity depletion was observed in patients of study group A-IV exposed to highest concentration of fluoride (12.01-16.00 mg/L). Post hoc Tukey's LSD multiple comparison test revealed that the activity of D1 enzyme was significantly (q= 0.32-0.58, 95% CI= -1.13 to 2.03; P<0.05- 0.01) lowered between and within the groups, as well as compared to the control group. The activity of D1 in patients of all fluoride exposed groups was found to be positively correlated with serum T3 levels.(r= 0.96, P<0.01).

The activity of D2 was declined significantly (F= 208.936, P<0.01) with concomitant increase in fluoride concentration (Figure 2). Post hoc Tukey's LSD multiple comparison test illustrated that the activity of D2 was significantly (q= 0.06- 0.56, 95% CI= -1.42 to 2.04; P<0.05- 0.01) declined in group A-III (8.01-12.00 mg/L) and A-IV (12.01-16.00 mg/L). However, the differences in study groups A-I and II was not statistically significant (p<0.82) when compared with control

Serum Thyroid Hormone Level:
Different effects were found depending on the concentration of drinking water fluoride. (Figure 3)
One way ANOVA (F=1875.023, p<0.0001) with Tukey's Kramer multiple comparison test illustrated that the level of TSH increased significantly (q=-11.96 to -25.40, 95% CI= -101.28 to 34.53,p<0.5 to 0.001) in fluorotic patients of all study groups as well as compared to control.

Serum T3 and T4 levels exhibited stepwise decrease in all the study groups in response to the concentration of drinking water fluoride. One way analysis showed a significant (f_{T3}=921.458, f_{T4}=432.348, p<0.0001) variance within and between groups. Post hoc Tukey's Kramer test revealed that the decline in serum levels of T3 and T4 was significant (T3, q=43.87 to 53.81, 95% CI= -162.42 to 192.24; T4 q=6.67 to 9.42, 95% CI= -199.62 to 147.85, p<0.05 -0.001) among the fluorotic study groups as well in comparison to control.

The level of FT3 and FT4 hormones also showed significant (F_{ft3}=157.89, p<0.01; F_{ft4}=205.671, p<0.001) variance. Post hoc multiple comparison test revealed significant (q_{ft3}=1.54-2.64, 95% CI= -8.08 -9.01; q_{ft4}=0.41 -0.75, 95% CI= -2.43 – 3.37, p<0.05 -0.001) decrease within the fluorotic groups as well as compared to control.

The mean serum level of protein bound iodine was significantly (f=278.702, p<0.001) lowered in all study groups (Figure 4). Post ANOVA analysis by Tukey's multiple comparison test illustrated a significant (q=0.98 to 1.86, 95%CI= -3.96 to 6.34,p<0.05-0.001) decrease among all the study groups as well as compared to control.

The concentration of fluoride in serum and urine was elevated significantly (F_{sf}=11.431,p<0.001;F_{uf}=1329.86,p<0.0001) in fluorotic patients affected with thyroid dysfunction in all the study groups. Tukey's LSD multiple comparison test revealed significant (q_{sf}=0.30to 0.56, 95%CI= -1.66 to 0.67, p<0.05 -0.001) increase in serum fluoride concentration among all the four study groups as well as compared to control group. Post hoc test analysis for urinary fluoride illustrated a significant (q_{uf}=-3.09 to-1.64,

95%CI= -10.37 to 4.95, p<0.05-0.01) alteration in all study groups, but in comparison to control there was a significant increase. As concentration of fluoride in drinking water increased, the concentration in serum was also increased but the excretion through urine was found to be decreased. It indicated the retention of fluoride in the body was increased.

Correlation Analysis:

We evaluated relationships between water fluoride, serum fluoride, thyroid hormones and activity of deiodinase enzymes among all the patients from different fluoride exposure. Pearson's bivariate correlation analysis showed inverse relationship between serum F and activity of D1 (r = -0.93, P<0.003,); and activity of D2 (r= -0.95, P<0.008) in all fluoride exposed groups(Figure 5). There was significant (P<0.002) negative relationship between serum F vs T3 (r = -0.84) and serum F vs T4 (r= -0.87, P<0.01) (Figure 6). Direct relationship was found between serum F and rT3 (r= 0.84, P<0.004) (Figure 7). The relationship among serum F and TSH (r= 0.52, P=0.76); serum F and FT3 (r=0.41, P=0.89) and serum F and FT4 (r=0.49, P=0.56) was statistically non significant.

Linear regression and correlation analysis revealed a significant (P<0.003) causal relationship between T3 and D1 (r = 0.96, P<0.002) (Figure 8). The regression equation indicates that increased water fluoride was a strong predictor for low levels of T3 (y= -0.139x + 0.981, R^2= 0.85) and low activity of D1 (y= - 11.46x + 111.3, R^2 = 0.67). The elevated levels of rT3 reveal a strong causal relationship with D1 (Figure 9). The low activity of D1(y=-0.139x + 0.981, R^2= 0.85) was a strong predictor of high levels of rT3 in fluorotic patients with different fluoride exposure.

Discussion:

The present study show that increased ingestion of fluoride in drinking water, produce alterations in thyroid function hormones (TSH, T3, FT3, T4, FT4, rT3), and changes in the activities of deiodinase enzymes. Among the three deiodinases, D1 is expressed in thyroid gland besides the liver and kidney, D2 is found in the brain, pituitary gland, and skeletal muscle, and D3 is highly expressed in brain, placenta and fetal tissue. Fluoride is known to interfere with the activity of the deiodinases (Susheela *et al*., 2005). The D1 activity in liver and kidney is stimulated in hyperthyroidism and decreased in hypothyroidism as a result of the positive feedback of T3 on D1 production (O'mara *et al*., 1993). The present study demonstrate significant (P<0.01) decline in T3 and T4 levels and low activity of D1 in fluorotic patients affected with thyroid hypofunction, which may be due to the important role of D1 in peripheral T4 to T3 conversion.

The large elevation in the level of rT3 with concomitant increase in fluoride concentration in drinking water in each study group, was well correlated with a repression of D1 activity. These alterations of D1 and rT3 levels were directly reflected on the expression the thyroid hormone (Eric *et al*.2007). Moreover, this expression of D1 suggests control of thyroid hormone activity via the alteration of the level of rT3. Reverse T3 has very low hormone activity and reports suggest that rT3 may decrease T3 activity by competing with T3 for binding to lipids, transporters and receptors affecting the activity of hormone in cell (Benvenga *et al*.1993; Mitchell *et al*.1999; Eric *et al*. 2007)

The level of D1 activity is inversely related to the level of rT3 in fluorotic patients affected with thyroid hypofunction. The findings are in consonance with the study of Schneider *et al*. (2006) in animals. Lin *et al*. (1991) found increased rT3 levels formed by excessive D3 activity in children. The balance of active T3 and inactive rT3 in the serum reflects thyroid hormone economy. It is also suggested that high fluoride might exacerbate the effects of iodine deficiency. In addition, a difference in T3/rT3 ratios between high- and low-fluoride area suggested that excess fluoride ion affects normal deiodination and the residents in endemic fluorosis areas (Susheela *et al*., 2005, Shashi and Singla, 2009) are afflicted with such physiological derangements.

In the present study a fluoride induced decrease in D1 activity, significantly affect T3 levels, suggesting that the conversion of T4 into T3 is affected as the fluoride concentration in water and in body fluids was increased and that extra hepatic tissues are unable to compensate for the lost T3 production in the liver through the activity of D2.

The location of D2 in the skeletal muscle provides another source of plasma T3 in addition to D1 conversion in the liver and kidneys. Because D1 is regulated by positive feedback and D2 is regulated by negative feedback mechanisms, their relative contributions to plasma T3 production may depend on thyroid state. D2 activity is significantly stimulated in Graves' disease via both TSH and TSH-receptor antibodies (Murakami *et al*., 2001). As in the present study, the activity of D2 was significantly lowered because fluoride has known effect on skeletal muscles (Shashi *et al*., 1992; Shashi and Sharma, 2011 (a) and resulting in lower levels of T3. Follicular thyroid carcinoma may express high levels of D2, resulting in highly elevated serum T3 levels (Kim *et al*., 2003; Shashi and Sharma, 2011(b).

Total T4 levels were significantly (p<0.01) decreased with increase in drinking water and serum fluoride concentration .which could be linked to the increase in rT3.One function of rT3 is to facilitate the removal of

excess T4 (Eric *et al.*2007). Increased rT3 in fluoride intoxication indicates that removal of rT3 is not functioning properly, thus prompting the decreased production of T4 in a hypothyroid state leading to an increase of TSH secretion by the pituitary gland, as observed in the present study, the level of TSH was significantly ($p<0.001$) elevated.
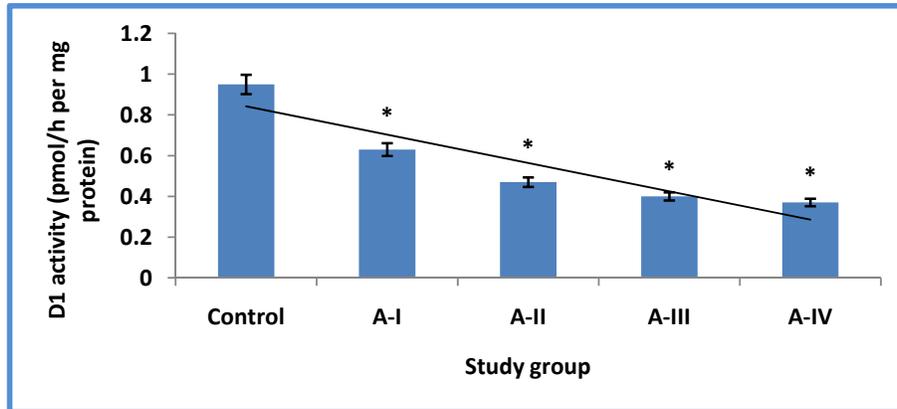
Fig. 1: Mean serum activity of D1 in fluorotic patients of all study groups
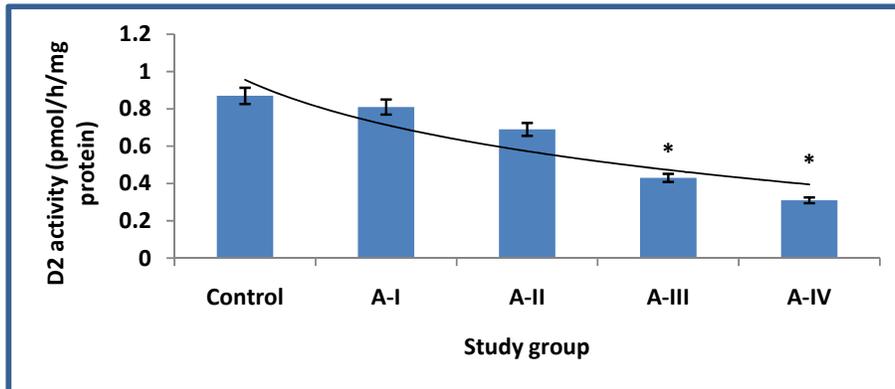
Fig. 2: Mean serum activity of D2 in fluorotic patients of all study groups
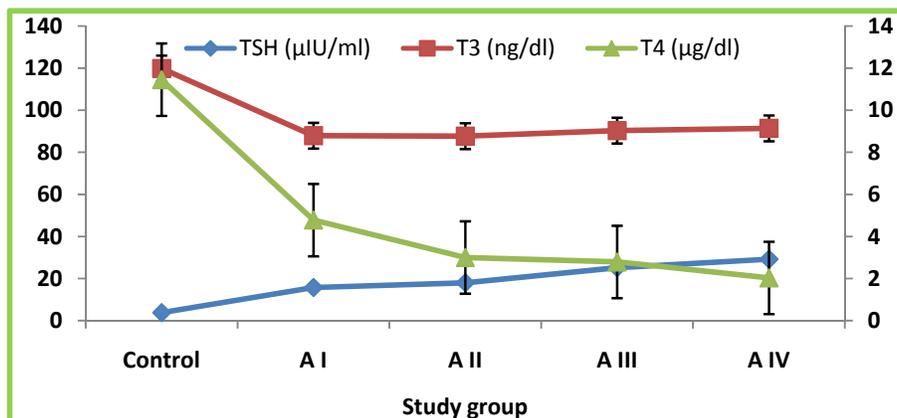
Fig. 3: Mean serum levels of TSH and thyroid hormones in fluorotic patients of all study groups
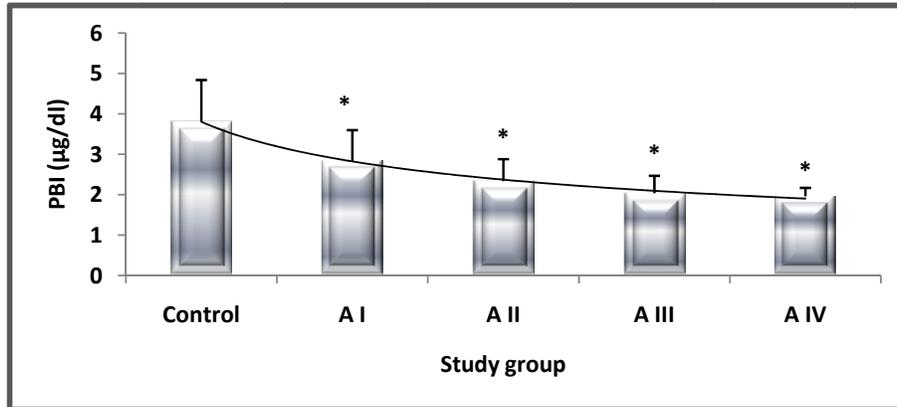
Fig. 4: Mean serum levels of Protein bound iodine (µg/dl) in control and fluorotic patients
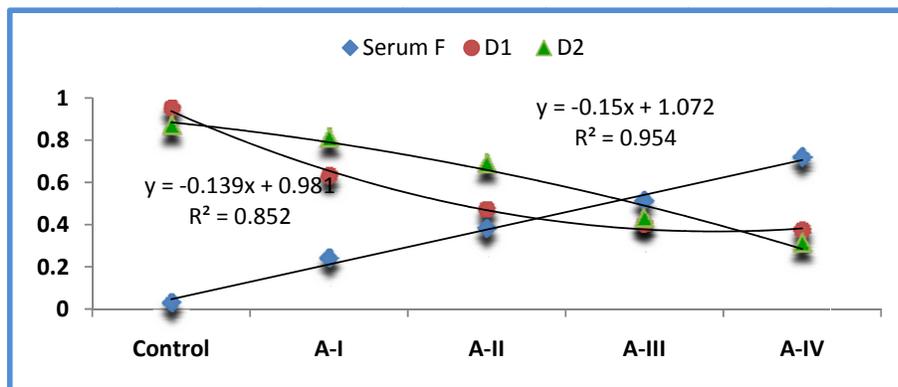
Fig. 5: Correlation and regression between serum F vs D1 and Serum F vs D2 activity in fluorotic patients of all study groups
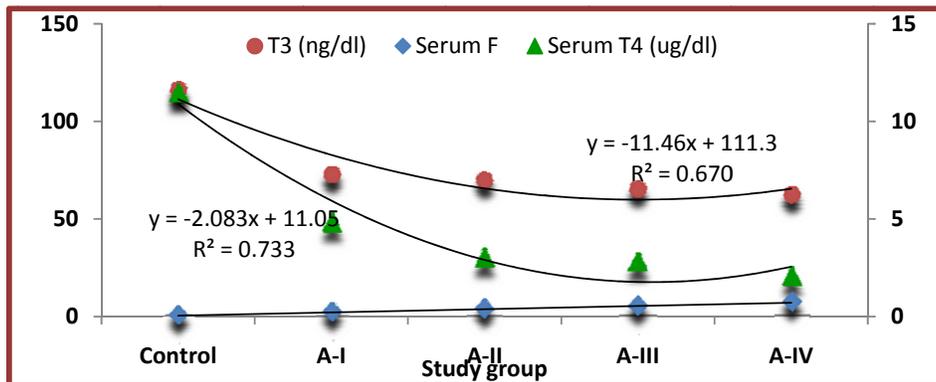
Fig. 6: Correlation and regression between serum F, T3 and T4 levels in fluorotic patients of all study groups
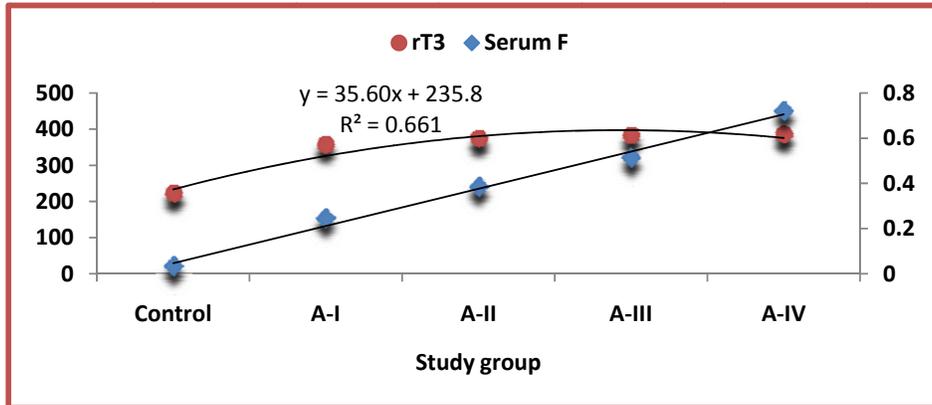
Fig. 7: Correlation and regression between serum F and rT3 levels in fluorotic patients of all study groups
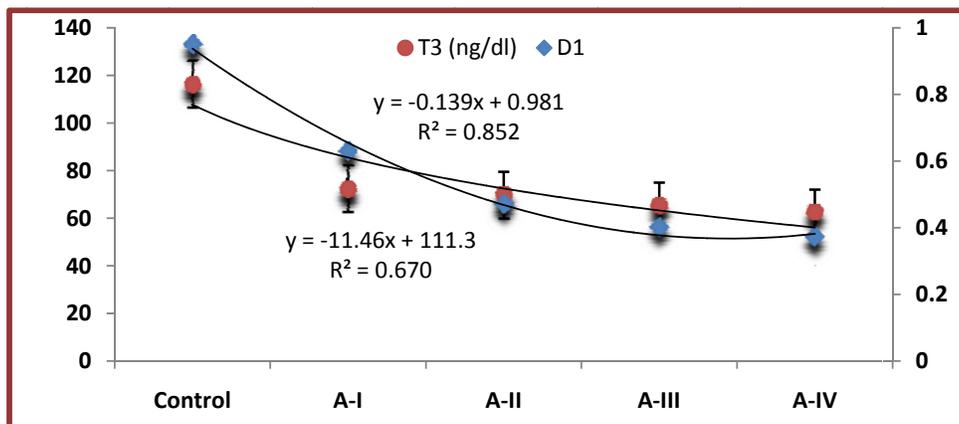
Fig. 8: Correlation and regression between serum T3 and D1 activity in fluorotic patients of all study groups
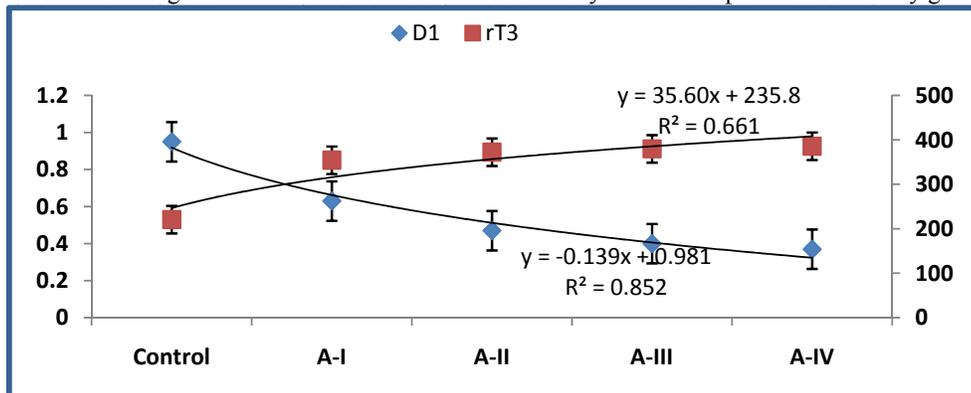
Fig. 9: Correlation and regression between serum rT3 and D1 activity in fluorotic patients of all study groups

Several sets of reported results are consistent with an inhibiting effect of fluoride on deiodinase activity; these effects include decreased plasma T3 with normal or elevated T4 and TSH and normal T3 with elevated T4 (Michael *et al*. 1996; Susheela *et al*. 2005; Shashi and Singla, 2009). The antihyperthyroid effect that Galletti and Joyet (1958) observed in some patients is also consistent with an inhibition of deiodinase activity in those individuals.

Together with the findings on D1 and D2 expression, it is suggested that this syndrome may be caused by high fluoride ingestion in drinking water, under normal iodine condition and lower levels of protein bond iodine. The lower levels of PBI may also due to chronic fluoride toxicity as reported by the other workers in experimental fluorosis (Bildik and Canas, 1996; Cinar and Selcuk, 2005) and in humans (Shivashankra *et al*.2000). A decrease in PBI, T3 and T4 levels in the blood is known to be associated with a decrease in the rate

of metabolism by as much as 30 to 40% in case of hypothyroidism (Guyton, 2008). The fact that an iodine deficiency caused by excessive fluoride intake and more excretion of iodine in the urine, by the reduced diet, protein malnutrition affecting iodine intestinal absorption, could be responsible for the changes observed here as reported in experimental animals (Lisboa *et al.*, 2003). The present study therefore provides evidence that fluoride in excess may be inducing diseases normally attributed to iodine deficiency.

Conclusion:

In conclusion, the present study demonstrate that nutritional factor modulate both 5'-deiodinase isoforms (D1 and D2). The lower activity of both D1 and D2 contribute to decrease in conversion of T4 toT3 and increased conversion of T4 to rT3. The depressed activity of D1 results in reduced conversion of rT3 into T2 and thereby allowing rT3 to accumulate in the body, resulting in hypothyroidism in fluoride intoxication.

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