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International Journal of Current Research
Vol. 9, Issue, 06, pp.51674-51681, June, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ASSESSMENT OF FLUORIDE STATUS IN PATIENTS WITH HYPOTHYROIDISM

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ARTICLE INFO

Article History:
Received 20th March, 2017
Received in revised form
14th April, 2017
Accepted 16th May, 2017
Published online 20th June, 2017

Key words:
Hypothyroidism,
Water,
Urine and Serum fluoride levels.

ABSTRACT

Introduction: Fluorosis an endemic problem affects many tissues including endocrine glands such as thyroid. Iodine deficiency remains the most common cause of primary hypothyroidism worldwide. Fluoride and iodine are both halogens. Fluoride, the negative ion of the element fluorine, easily displaces iodine in the body because it is much lighter and therefore more reactive. Few studies have reported no relationship between serum fluoride levels and thyroid disorders, while others have reported decreased serum thyroid hormone levels are associated with increased fluoride levels in hypothyroidism. Goiter and hypothyroidism are known to be caused by iodine deficiency. However, it has also been found to occur in areas where there are adequate supplies of iodine, but where there is an excess fluoride in the water. The present study was conducted to assess the fluoride status in newly diagnosed cases of hypothyroidism.

Aims & objectives

1)To assess the fluoride status in the patients of hypothyroidism and healthy controls.

2)To correlate the fluoride status with the severity of hypothyroidism.

Material & Methods: The present study was conducted in the Department of Biochemistry in collaboration with Department of Medicine (Endocrinology), Pt. B.D. Sharma PGIMS, Rohtak. Fifty newly diagnosed patients with hypothyroidism in the age group 20-55 years and fifty age and sex matched healthy controls were taken in the study. Fasting venous blood samples were collected from cases and controls for routine biochemical, hormone analysis and fluoride estimation after obtaining informed written consent and complete history.

Results: Mean water fluoride level of cases was 2.308 ± 0.282 ppm (Mean ± SE) and of controls was 1.659 ± 0.183 ppm and found to be *statistically significant.* Mean value of serum fluoride levels in study group was 0.234 ± 0.023ppm and of controls was 0.058 ± 0.007ppm and found to be *statistically significant.* Mean urine fluoride level of cases was 2.359 ± 0.146 ppm and of controls was 1.577 ± 0.166 ppm and found to be *statistically significant.* TSH in the study group (20.924 ± 2.398) and control group (2.817 ± 0.186) were compared and found to be *statistically significant.*

Discussion & Conclusion- In the present study the serum & urinary fluoride levels in the study & control groups indicate the likelihood of fluoride ingestion from food & other sources is apparently, like dental products, cosmetics etc. It is evident in consistent with the findings of reported studies that analysis of fluoride in body fluids besides drinking water is highly relevant & necessary for understanding & prevention of potential health implications like hypothyroidism seen in endemic areas.

Citation: Dr. Manju Bala Pahwa, Dr. Parveen, Dr. Rajesh Rajput and Dr. Meenakshi, 2017. "Assessment of fluoride status in patients with hypothyroidism", *International Journal of Current Research*, 9, (06), 51674-51681.

INTRODUCTION

Fluorosis continues to be an endemic problem. To certain extent (0.6 ppm) fluoride ingestion is useful for bone and teeth development, but excessive ingestion causes a disease known as fluorosis, which was earlier considered to be a problem related to teeth only, has now turned up to be a serious health hazard affecting many systems of body including endocrine glands such as thyroid (Carton and Park, 2006). The global goitre prevalence is more than two billion with more than fourty billion in India (Abraham *et al*., 2009). In India population based study done in cochin on 971 adults showed 3.9% of prevalence of hypothyroidism (Zhu and Cheng, 2010).

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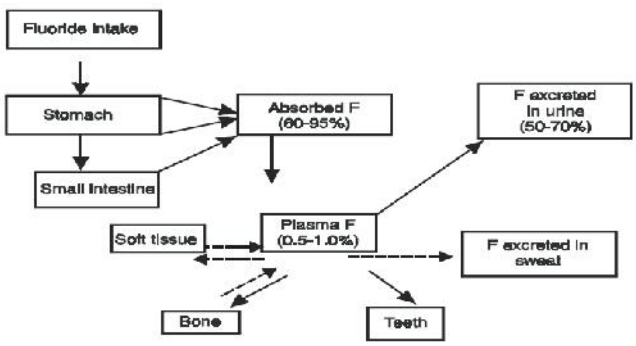
Fluorosis is a global problem affecting more than seventy million people in twenty five countries. In India about 62 million people are consuming excess of fluoride in drinking water (Susheela, 1999). The problem of excessive fluoride in ground water in India was first reported in 1937 in the State of Andhra Pradesh (Short *et al*., 1937). In India the problem has reached alarming proportions affecting at least 20 out of the 36 states and union territories of Indian Republic. In Haryana, total number of 12 districts endemic for fluorosis are Rohtak, Bhiwani, Faridabad, Gurgaon, Jind, Karnal, Kurukshetra, Kaithal, Mohindragarh, Sonipat, Sirsa and Rewari (Fluorideandfluorosis.com. New Delhi: Fluorosis research and rural development foundation online resource). Thyroid hormones are of vital importance in regulating the wide array of metabolic parameters (Prasad and Kumar, 2005). Hypothyroidism results from under secretion of thyroid hormones from the thyroid glands (Fluorideandfluorosis.com.

New Delhi: Fluorosis research and rural development foundation online resource). It is characterized by low serum thyroid hormone levels and is associated with reduced metabolism, reduced lipolysis, weight gain, reduced cholesterol clearance and elevated serum cholesterol (Thyroid Guidelines Committee (2002)). Iodine deficiency remains the most common cause of primary hypothyroidism worldwide. A sensitive thyroid stimulating hormone (TSH) assays should always be used as an initial screening test to establish a diagnosis of primary hypothyroidism. Additional test may include the estimation of total and free triiodothyronine (FT_3) and free thyroxine (FT_4), thyroid autoantibodies (anti thyroperoxidase and anti-thyroglobulin autoantibodies) (Longo *et al*., 2008; Utiger, 1989; Becker *et al*., 1993). Fluoride and iodine are both halogens. Fluoride, the negative ion of the element fluorine, easily displaces iodine in the body because it is much lighter and therefore more reactive. In addition to dental hygiene products and drinking water, many breakfast cereals, juice concentrates, soda, tea and other processed foods contain alarming levels of fluoride (Burgstahler and Spittle, 2007). The recommended level of fluorides in drinking water in India is less than 1 ppm (Sendesh and Ramasubramanian, 2011). Fluoride is rapidly absorbed from GIT with a half-life of 30 minutes. After a single dose plasma concentration rise to a peak then fall as the fluoride is cleared by the renal system and bone, decreasing that to short term baseline with a half-life of several hours. Fluoride concentrations in plasma, extracellular fluids and intracellular fluids are in approximate equilibrium. The concentrations in the water of most tissues are thought to be 40% to 90% of plasma concentrations. Fluoride is cleared from plasma through two primary mechanisms: uptake by bone and excretion in urine. Plasma clearance by the two routes is approximately equal in healthy adult humans. The relative clearance by bone is larger in younger ones and children because of their growing skeletal systems. Renal clearance depend upon pH and glomerular filteration rate. At low pH, more hydrogen fluoride (HF) is formed promoting reabsorption (Fluoride in Drinking Water: A Scientific Review of EPA's Standards, 2006).



Goiter and hypothyroidism are known to be caused by iodine deficiency. However, it has also been found to occur in areas where there are adequate supplies of iodine, but where there is an excess fluoride in the water. In humans, effects on thyroid function were associated with fluoride exposures of 0.05-0.13 mg/kg/day when iodine intake was adequate and 0.01-0.03 mg/kg/day when iodine intake was inadequate. (12=23) This simply means that for a 70 kg person (often called the "standard man"), fluoride doses as low as 3.5 mg/day for those with an adequate intake of iodine, and 0.7 mg/day for those with an inadequate intake of iodine may have effect on the thyroid (Carton and Park, 2006). In 2005, Shusheela and coworkers concluded in their study that the children with dental fluorosis living in endemic fluorosis area and iodine deficiency disorders may have thyroid derangement that require special care and attention. However, the primary cause of Iodine deficiency disorders (IDD) may not always be iodine deficiency. But it might be induced by fluoride poisoning (Susheela *et al*., 2005). In 2009, Xiang and coworkers did study and confirmed that the high fluoride exposure can cause functional abnormalities of thyroid and the different severity degree of dental fluorosis may be related to significant deviation in the serum levels of thyroid hormones (Xiang *et al*., 2009). In a study by Hosur and coworkers in 2012 did not show any significant alteration in the levels of thyroid hormones FT_3, FT_4 and TSH in subject with dental fluorosis (Hosur *et al*., 2012). In the views of controversial studies done mostly in growing children (10-14 years) regarding whether the fluoride status affects the thyroid gland function significantly or not especially in adults; the present study was planned to determine the fluoride status and its influence on thyroid hormones level in patients of hypothyroidism.

Aims and Objectives

1) To assess the fluoride status in the patients of hypothyroidism and healthy controls.
2) To correlate the fluoride status with the severity of hypothyroidism.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry in collaboration with Department of Medicine (Endocrinology), Pt. B.D. Sharma PGIMS, Rohtak.

Inclusion criteria

Fifty newly diagnosed patients with hypothyroidism in the age group 20-55 years and fifty age and sex matched healthy controls were taken in the study.

Exclusion criteria

Any history suggestive of total/ subtotal throidectomy, patients on I^{131} treatment, lithium, antithyroid drugs and diagnosed cases of Grave's disease, toxic multinodular goiter, toxic adenoma, carcinoma, gestational hypothyroidism, chronic renal disease and history of radiation exposure. After getting written informed consent from the cases and controls, detailed history were obtained and recorded in their respective proforma. They were subjected to physical examination and anthropomrtric measurements as per protocol were taken followed by systemic examination and routine and special investigations were performed. Fasting venous blood sample (5mL) was taken from the antecubital vein aseptically in plain plastic red vacutainer. Sample was processed within one hour of sample collection. Serum was separated by centrifugation at 2000 rpm for 5 minutes after clotting.

FT_3, FT_4 and TSH were estimated by two-site sandwich immunoassay using chemiluminometric technology (Advia Centaur'CP immunoassay system) (Burtis *et al*., 2006). Serum fluoride levels was estimated by using 'Thermo Scientific Orion fluoride ion selective electrode and Thermo Scientific Orion ISE meter' (Fig 1) (Shusheela, 2007; Hall *et al*., 1972).

Figure 1. Five point calibration curve

RESULTS AND OBSERVATIONS

The mean ages of the cases was 35.5 ± 10.01 years. The mean age of control group was 34.74 ± 9.53 years. In the study group 8% of patients were males and 92% of patients were females. In control group 12% persons were males and 88% persons were females. (Figs 2&3) According to rural and urban distribution of cases 36 patients were from rural area, which constitutes 72% of total cases and 14 patients were from urban area accounting for 28% of total cases. In the controls group 29 persons were from rural area i.e. 58% of total controls and 21 persons were from urban area 42% of total controls. (Fig 4&5) According to source of drinking water, in the study group 19 (38%) patients were using municipality drinking water supply and 31 (62%) were using drinking water from ground water source. In the control group 23 (46%) persons were using municipality drinking water supply and 27 (54%) were using drinking water from ground water source. (Fig 6&7) Mean water fluoride level of cases was 2.30 ± 0.282* and of controls was 1.65 ± 0.183* and found to be *statistically significant*. Mean value of serum fluoride levels in study group was 0.234 ± 0.023* and of controls was 0.058 ± 0.007* and found to be *statistically significant*. Mean urine fluoride level of cases was 2.35 ± 0.146* and of controls was 1.57 ± 0.166* and found to be *statistically significant*. (Table I &II) TSH in the study group (20.92 ± 2.39*) and control group (2.81 ± 0.18*) were compared and found to be *statistically significant*. (Table III) TSH levels in the study group were positively and significantly correlated with serum fluoride levels. There was negative insignificant correlation between FT_4 & serum fluoride levels, but correlation of FT_3 was positive insignificant. (Fig 11) In the study group 28 patient's TSH was in range of 10.01 to 30.00 and 12 patients were having FT_4 in normal range. Most of them were having increased serum fluoride and urine fluoride levels. None of the patient in the study group was having FT_3 in the normal range. All of the parameters of thyroid hormone levels in control group were in normal reference range. (Table III)

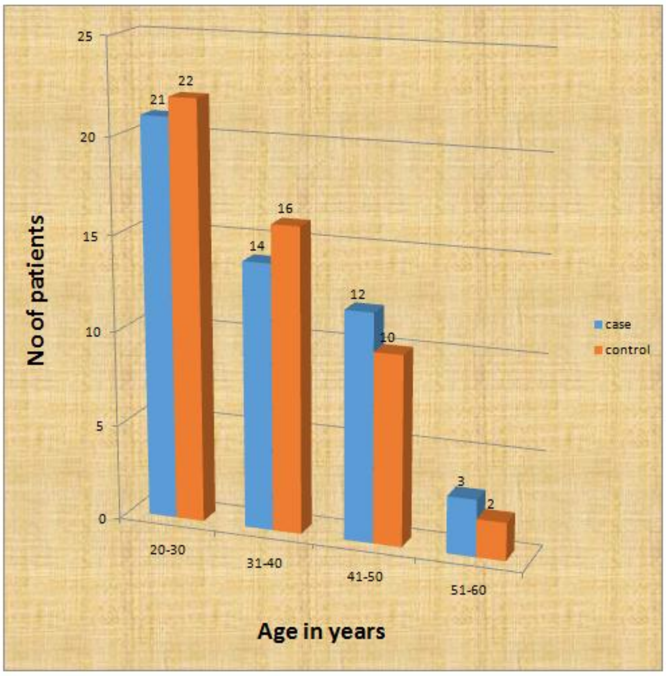


Fig.2. Age distribution of cases and controls

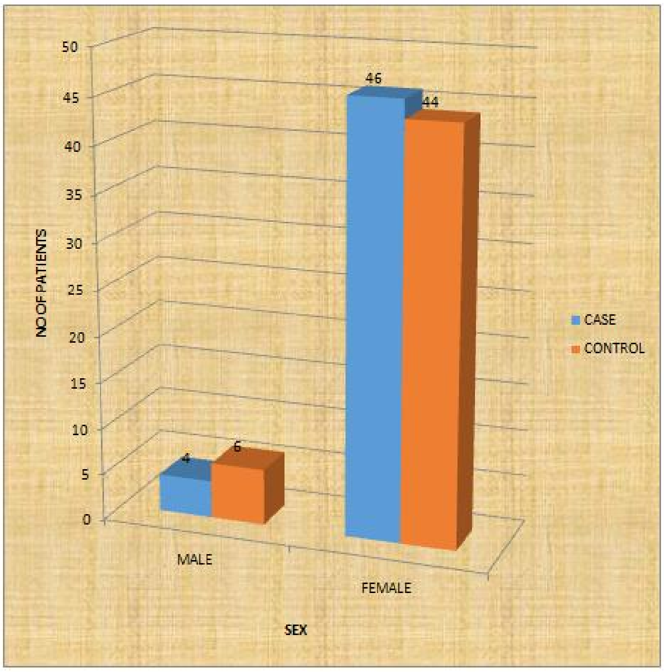
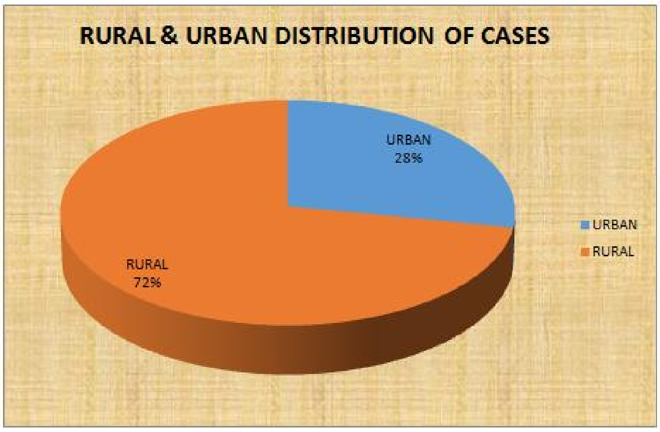


Fig.3. Sex distribution of cases and controls



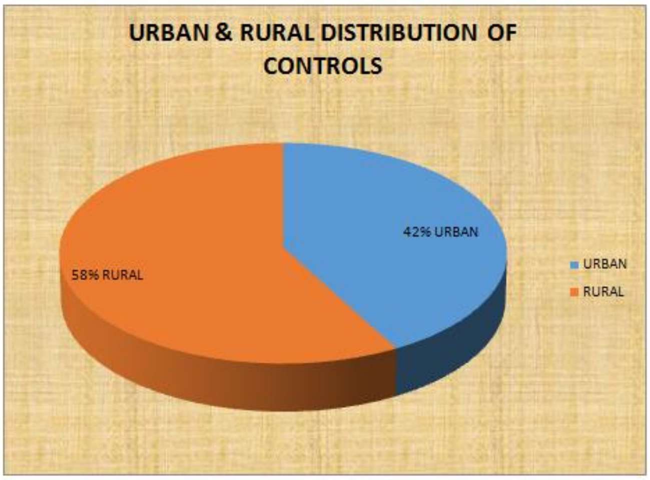
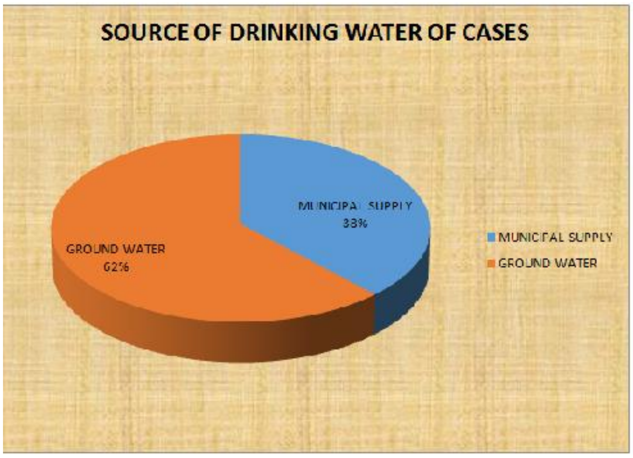


Fig.4 &5



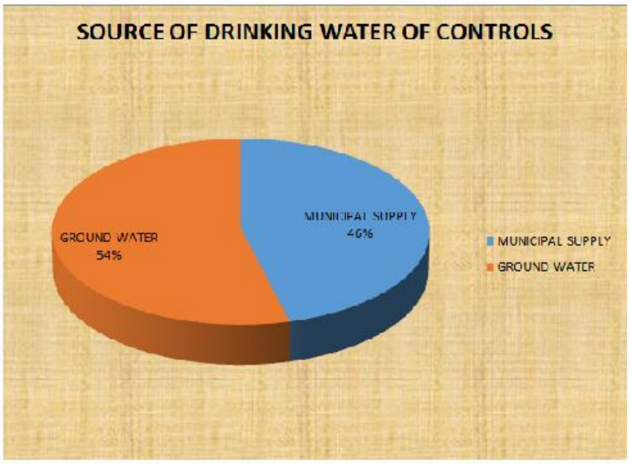


Fig.6&7

Table I

Age in years	Cases	Controls
20-30	21(42%)	22(44%)
31-40	14(28%)	16(32%)
41-50	12(24%)	10(20%)
51-60	3(6%)	2(4%)

The age distribution of cases and controls is shown in the table 1. The age group of the persons ranged from 20-55 years. The mean age of the cases was 35.3 ± 10.01 years. The mean age of control group was 34.74 ± 9.53 years.

Table II

AREA	CASES	CONTROLS
RURAL	36(72%)	29(58%)
URBAN	14(28%)	21(42%)

According to rural and urban distribution of cases 36 patients were from rural area, which constitutes 72% of total cases and 14 patients were from urban area accounting for 28% of total cases. In the control group 29 persons were from rural area i.e. 58% of total controls and 21 persons were from urban area 42% of total controls.

Table III. Distribution of patients according to source of drinking water

Source	Cases	Controls
Municipal supply	19(38%)	23(46%)
Ground water	31(62%)	27(54%)

Table IV. Statistics of study group

Parameter	Mean	Std. error	Std. deviation
Age	35.30	1.41	10.01
FreeT3	1.661	0.056	0.398
Free T4	0.750	0.036	0.259
TSH	20.924	2.398	16.962
Serum fluoride	0.234	0.023	0.166
Water fluoride	2.308	0.282	1.999
Urine fluoride	2.359	0.146	1.033

Table V. Statistics of control group

Parameter	Mean	Std. error	Std. deviation
Age	34.74	1.34	9.53
FreeT3	3.053	0.057	0.403
Free T4	1.163	0.027	0.199
TSH	2.817	0.186	1.320
Serum fluoride	0.058	0.007	0.054
Water fluoride	1.659	0.183	1.298
Urine fluoride	1.577	0.166	1.178

Table VI. Comparison of water fluoride levels in cases and controls

Water fluoride range(ppm)	Cases	Controls
Safe 0-1.0*	23(46%)	29(58%)
Marginally contaminated 1.1-3.0	13(26%)	15(30%)
Unsafe	14(28%)	06(12%)
3.1-5.0	09	06
5.1-6.0	03	0
≥6.1	02	0

*Normal range of fluoride in water (0-1.0 ppm)

Fluoride content of drinking water of 23 patients in the study group was up to 1.00 ppm (safe limit), out of which 17 were using municipality watersupply, 6 patients were using drinking water from underground sources categorized as safe. 13

patients were using water with fluoride content of 1.01-3.00 ppm, categorized as marginally contaminated, out of 13 only one patient was using water from municipal supply. Rests of the patients were using water with fluoride content > 3.00 ppm which was categorized as unsafe for drinking purposes.

The numbers of persons using safe water were 29 in control group, out of which 23 patients in control group were using safe drinking water from municipal supply and 6 patients were using water from underground sources having levels in safe range of ≤ 1 ppm. 15 patients in the control group were using marginally fluoride contaminated water from underground sources. Rest of the persons in the control group were using water with fluoride content > 3.01 ppm which was categorized as unsafe for drinking purposes.

Table VII. Comparison of serum fluoride levels in cases and controls

Serum fluoride range(ppm)	Cases	Controls
0.010-0.020*	06	14
0.021-0.100	09	26
0.101-0.200	08	09
0.201-0.400	17	01
0.401-0.600	09	0
≥ 0.601	01	0

*Normal range of fluoride in serum (0.010-0.020 ppm)

Table VIII. Urine fluoride levels comparison of urine fluoride levels in cases and controls

Urine fluoride range(ppm)	Cases	Controls
0.000-0.100*	0	04
0.101-1.000	05	19
1.010 – 4.000	41	26
1.010-2.000	20	13
2.010-3.000	10	08
3.010-4.000	11	05
≥4.010	04	01

Table IX. Comparison of thyroid hormone status in cases and controls

	CASES (mean ± std. error)	CONTROLS (mean ± std. error)
TSH * (0.35-5.5 µmol/l)	20.92 ± 2.39	2.81 ± 0.18
FT3 *(2.3-4.2 pg/ml)	1.66 ± 0.056	3.05 ± 0.057
FT4 *(0.89-1.76 ng/dl)	0.75 ± 0.036	1.16 ± 0.027

*Indicates normal range

Table X. Fluoride content of drinking water, urine, and serum in the study group

Total no cases investigated	Age range (years)	Range of fluoride content (ppm)		
		Drinking water	Serum fluoride	Urine fluoride
50	22-55 (35.30)	0.130-9.100 (2.30)	0.012-0.661 (0.23)	0.928-4.680 (2.35)

Table XI. Fluoride content of drinking water, urine, and serum in the control group

Total no persons investigated	Age range (years)	Range of fluoride content (ppm)		
		Drinking water	Serum fluoride	Urine fluoride
50	20-55 (34.37)	0.09-4.50 (1.65)	0.01-0.29 (0.058)	0.09-4.15 (1.57)

Table XII. Correlation of water fluoride levels with serum fluoride and urine fluoride

	Parameter	r value	p value
Water fluoride	Serum fluoride	0.272	0.056
	Urine fluoride	0.545*	0.01

*Correlation is significant at the 0.01 level (2-tailed)

Levels of water fluoride and serum fluoride when correlated by Pearson's Correlation were found to be positively correlated but statistically insignificant. But correlation of water fluoride with urine fluoride is found to be statistically significant.

P.S : Kindly see the attachments for figures and scanned signatures in mail sent to you.

Fluoride content of drinking water of 23 patients in the study group was up to 1.00 ppm (safe limit), out of which 6 patients were using drinking water from underground sources categorized as safe. The number of persons using safe water was 29 in control group. The data also reveal that they have higher than normal fluoride content in their body fluids. (Table IV) The serum levels of the study group are in 0.012-0.661 ppm range and 0.01-0.29 ppm in control group. Percentage of persons with serum fluoride levels between 0.010-0.020 ppm (normal upper limit) were 12% in study group and 28% in control group. (In the study group there was no patient having urine fluoride level in normal range.

But in the control group urine fluoride level of 4 patients (8%) was within normal range. (Fig 8-10) Levels of water fluoride and serum fluoride when correlated by Pearson's Correlation were found to be positively correlated but statistically insignificant. But correlation of water fluoride with urine fluoride was found to be statistically significant. (Fig 12 & 13)

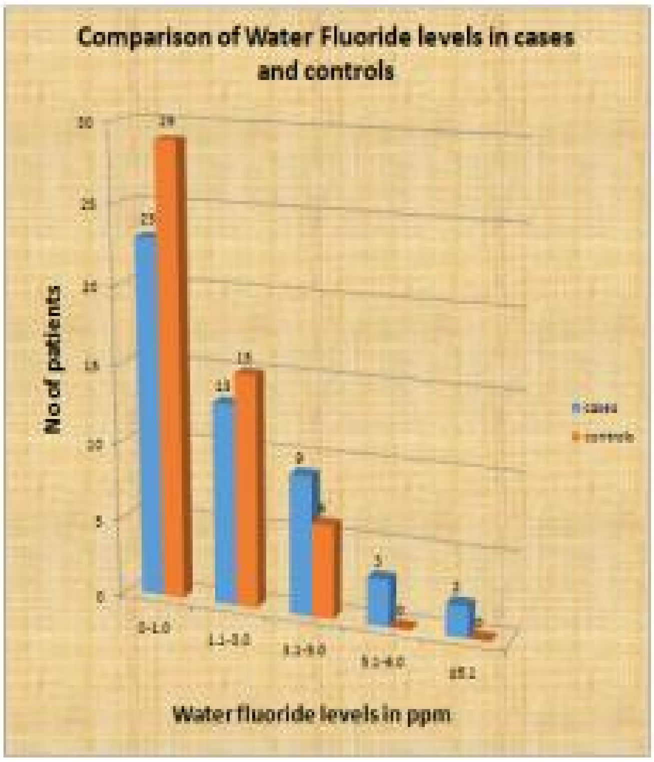


Fig.8

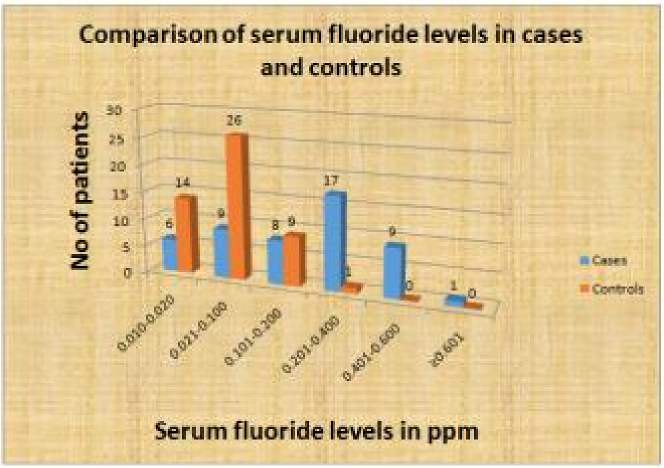


Fig.9

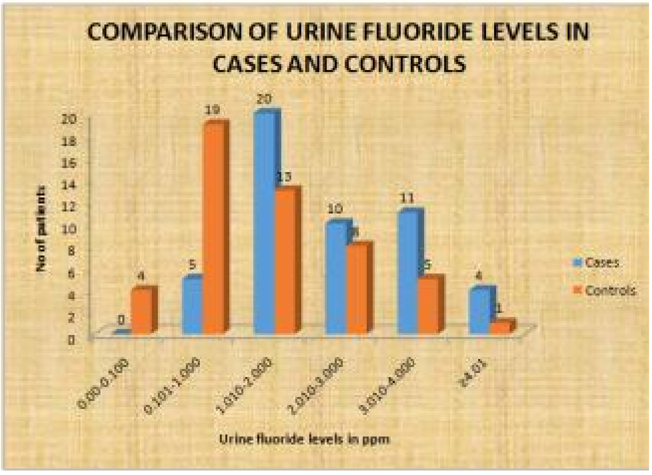


Fig.10

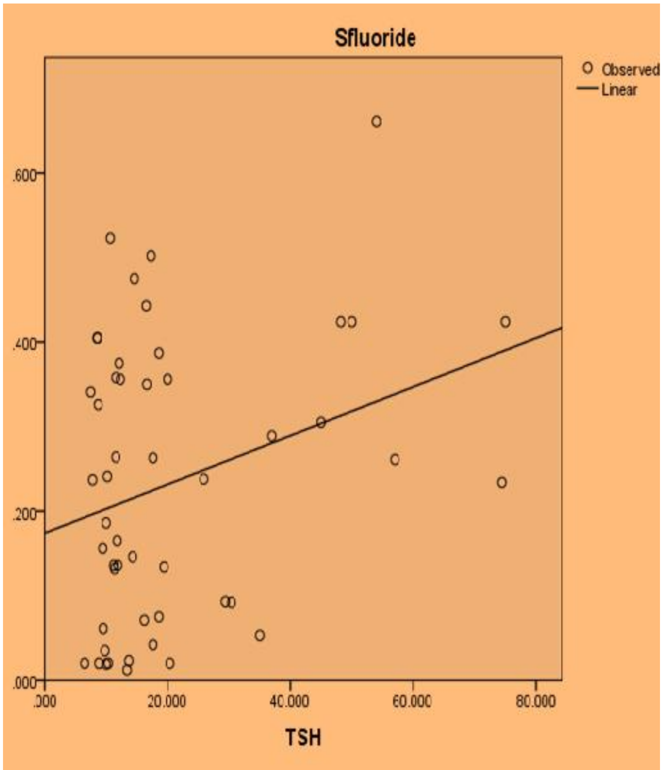


Fig.11. Graph showing correlation of serum fluoride with TSH

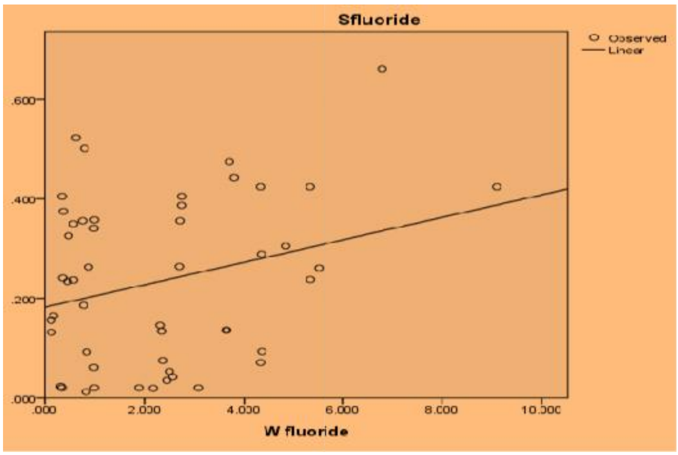


Fig.12. Graph showing correlation of water fluoride with serum fluoride

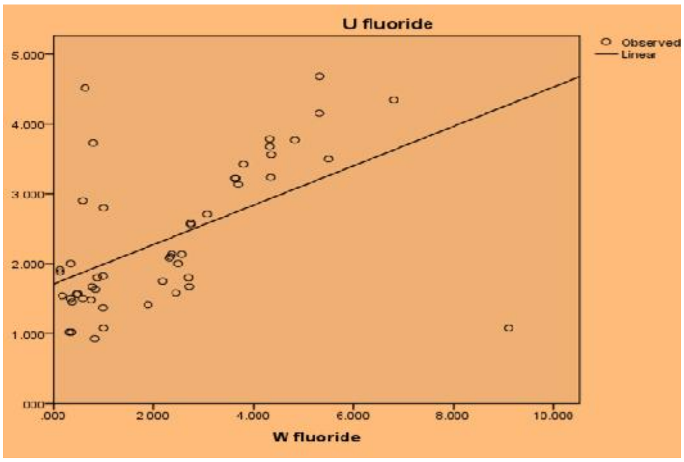


Fig.13. Graph showing correlation of water fluoride with urine fluoride

DISCUSSION

Although it has long been suggested, that high fluoride in drinking water/fluorosis was associated with iodine deficiency disease and dysfunction of thyroid gland (Carton and Park, 2006; Wilson, 1941; PFPC. History of the fluoride / iodine antagonism 2002; Pighini, 1923; Steyn, 1948; Steyn, 1955; Siddiqui, 1969; Day and Powell Jackson, 1972; Waldbott *et al.*, 1978; Jooste *et al.*, 1999). This study to our knowledge, is the first to investigate serum fluoride levels in adults in relation to TSH and the thyroid hormones FT_3 and FT_4 in patients with hypothyroidism. The fluoride is a universal G-protein activator/ inhibitor. The stimulation of certain G-proteins occurs due to toxic effects of fluoride, which has the effects of switching off the uptake into the cell of the active thyroid hormone. The thyroid control mechanism is compromised (PFPC. History of the fluoride / iodine antagonism 2002). Fluoride competes with TSH for the receptors sites on the thyroid gland, so that less of this hormone reaches the thyroid gland and so lesser FT_3 and FT_4 hormones are synthesized (Tezelman *et al.*, 1994). Production of thyroid hormones is regulated by negative feedback mechanism, i.e., when the pituitary gland senses a drop in FT_3 levels in circulation, it releases more TSH to stimulate the thyroid gland which in turn accelerate the production of thyroid hormone T_4, now considered a “pro-hormone” (Longo *et al.*, 2008). The major source of circulating T_3 in blood is peripheral deiodination of T_4 and not from thyroid secretion (Stolc and Podoba, 1960). The enzymes which catalyze deiodination are called iodothyronine deiodinases, of which three have been identified, namely D1, D2 and D3, of which a brief discussion is relevant to be abnormalities detected in the present study. D1 activity is known to be responsible for conversion of T_4 to T_3 in peripheral tissues, particularly in the liver, and is reflected in plasma T_3 levels, subject of further investigation. D1 has both outer ring deiodination (ORD) as well as inner ring deiodination (IRD) activity. D2 activity reflects conversion of T_4 to T_3 in target tissues (local). This deiodinase has only ORD activity. However, the recent identification of D2 in human skeletal muscle supports the view that part of plasma T_3 may be generated from tissues other than liver. D3 activity, on the other hand, converts T_4 into the metabolite reverse T_3 (rT_3). D3 has only an IRD activity and is an inactivating enzyme. 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 tissues. (Gordonoff and Minder, 1960) While our investigations have focused on thyroid hormones rather than the deiodinases, fluoride is known to interfere with the activity of the deiodinases (Gordonoff and Minder, 1960; Gorlitzer von Mundy, 1963; Mikhailets *et al*., 1996).

Our findings further strengthen the possibility that fluoride is often responsible for thyroid hormone alteration normally ascribed to iodine deficiency disease (IDD). Iodine supplementation for control of IDD is widely practiced all over the India as a well as in certain other countries where fluorosis is endemic (Assessment of iodine deficiency disorders and monitoring their elimination: a guide for programme managers; Tyabji, 1990). A level of 15.0 ppm iodine was tested in the salt consumed and was considered satisfactory (Kapilashrami and Mathiyazhagon, 2003). The present study therefore provides evidence that fluorosis in excess may be inducing diseases normally attributed to iodine deficiency. Fluoride itself has been effectively used as an anti-thyroid drug (Galletti and Joyet, 1958). Adverse effect of excessive fluoride ingestion on the intelligence of children born to mothers in China living in endemic areas of fluorosis and iodine deficiency throughout the period of pregnancy indicate that the deleterious effects are especially critical on brain tissue if exposure occurs very early in development (Trivedi *et al*., 2007; Seraj *et al*., 2006; Zhao *et al*., 1996; Xiang *et al*., 2003). In view of the serum and urinary fluoride levels in the study and control groups in the present study, wherein the likelihood of fluoride ingestion from food and other sources is apparent, a good number of reports from India highlighting high fluoride intake through food and dental products have appeared (Han *et al*., 1995; Gupta *et al*., 1991; Rajan *et al*., 1987). It is evident therefore, that analysis of fluoride in body fluids besides drinking water is highly relevant and necessary for understanding potential health implications. Results of the present investigation indicate that better improvement in the health of the persons residing in fluoride endemic area, would likely be achieved if management strategies incorporate emerging knowledge to address both fluoride toxicity and iodine deficiency in the same individual, even if residing in non- iodine deficient areas. Iodine supplementation may not be adequate to reduce IDD when excess fluoride is being consumed. Detailed information on intake of fluoride including other sources like bottled water, cosmetics, food items etc. need to be taken into account as these items do contribute to fluoride levels in body fluids & removal of fluoride from ingestion needs to precede iodine supplementation.

Interests of Conflicts

There is no area of conflict in this research project.

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