Journal of Research in Medical and Dental Science
2023, Volume 11, Issue 1, Page No: 76-81

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Soil Iodine, Urinary Iodine and Thyroid Hormone Profiling of Ethnic Population of Eastern Himalayan Range of India

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ABSTRACT

This study was undertaken among two ethnic races living in rural, hilly terrain of Eastern Himalayan range with the hypothesis that depleted soil iodine content may be the cause of thyroid disorder in the tribal population. The study randomly selected individuals out of which 49.8% are male and 50.2% are female ranging from 10 to 90 years.

Method: The iodine content in soil and urinary iodine content (UIC) was analyzed along with anti-TPO antibody (anti-TPO Ab) assay and thyroid profiling. Soil and UIC were determined by arsenic cerium catalytic spectrophotometry method by Sandell-Kolthoff reaction. The anti-TPO Ab was determined using Electrochemiilluminiscence immunoassay analyzer. Thyroid hormone analysis was carried out by chemiluminescence assay.

Results: The average value of soil iodine content was 2.89 mg kg-1. The median UIC was 111.78 µg/l. Incidence of thyroid disorder was found to be 27.09 %. The distribution of hypothyroid and hyperthyroid was 24.85% and 2.35% respectively in the studied population out of which 66.4% were female are 33.57% male. 12 % of population showed high anti- TPO Ab which may be a cause of thyroid disorder. A negative correlation was observed between UIC and anti- TPO Ab (r=-0.078, P=<0.05)

Conclusion: The work is an attempt to demonstrate how the soil iodine status influences thyroid functioning among the under-represented ethnic population of Eastern Himalayas which is manifested as high prevalence of subclinical hypothyroidism (SCH) with iodine deficiency. The work underscores region specific recommendation on iodine supplementation for populations living in geographically remote locations.

Key words: Soil iodine, Ethnic race, Urinary iodine, Thyroid hormone, Anti-TPO antibody

HOW TO CITE THIS ARTICLE: Trisha Sonowal, Jatin Sarmah, Manab Deka, Paresh Kumar Sarma, Soil Iodine, Urinary Iodine and Thyroid Hormone Profiling of Ethnic Population of Eastern Himalayan Range of India, J Res Med Dent Sci, 2023, 11 (1):76-81.

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Received: 31-December-2022, Manuscript No. jrmds-22-78716;

Editor assigned: 02-January-2023, PreQC No. jrmds-22-78716(PQ);

Reviewed: 16-January-2023, QC No. jrmds-22-78716 (Q);

Revised: 20-January-2023, Manuscript No. jrmds-22-78716(R);

Published: 27-January-2023

EDITORIAL

Iodine is an essential micronutrient required for production of thyroid hormones, which regulate multiple biochemical processes and metabolism [1]. Deficiency of iodine in human diet causes abnormal consequences collectively called iodine deficiency disorders (IDD) [2]. Its deficiency includes hypothyroidism, goiter, mental retardation, decreased fertility, infant mortality etc. Increased thyroid hormone synthesis leads to hyperthyroidism which results in autoimmune disorders. For proper functioning of the thyroid gland, iodine has to be sufficient in the diet. The WHO recommended amount of daily iodine intake is 150-200 µg/l for adults, 90-120 µg/l for children and 250 µg/l for pregnant and lactating mothers [3]. About 70-80% of iodine requirement of human comes from vegetable sources [2]. The concentration of iodine in vegetables is determined by iodine content in soil and their biological assimilation. Iodine content in soil is relatively lower in mountainous areas due to runoff of top soil, which results in prevalence of IDD [4].

In India, IDD control program was initiated in 1962 as National Goiter Control Program (NGCP) with the objective to cover a wide range of IDD like mental and physical retardation, cretinism, still-births, abortion by following WHO declaration, 1957. Nearly 91 percent of households in the country have access to iodized salt

with 71 percent consuming adequate iodized salt, yet 42 million people in India suffer from different thyroid disorders [5]. Worldwide data have shown that the spectrum of thyroid disease varied in inland and coastal regions with hypothyroidism predominantly in inland and thyroid nodules in coast [6]. In a country like India where vast altitudinal variation exists from high mountainous Himalayan region in the north to the Arabian sea in the south necessitates a region-specific study on soil iodine availability and its influence in IDDs. Again; the data on iodine nutrition on the tribal population living in remote locations from in mountainous north eastern region of India is substantially weak to contribute towards nationally representative data of India.

The association between thyroid function and race is established by comparing Black and White people of the US where Black people have lower prevalence of hypothyroidism than the Whites [7].

This study is conducted in the moderate altitude (1,683ft msl) Dima Hasao district of Assam, India in eastern Himalayan goitrogenic belt. Heavy rainfall during monsoon and soil surface run off leads to degradation of iodine in the soil. The region represents linguistically unique admixture of diverse indigenous communities. The major communities and their total population in the district are Dimasa (74,502), Kuki (24,079), Naga (20,832) and Hmar (15,070). The minor ethnic communities are Karbi, Biate, Sonowal-Kachari and Garo of Mongoloid race and tea tribe (1,722) of Proto-Australoid race. The total population of the district is 2, 14, 102. The study was undertaken to demonstrate the status of soil iodine in the remote, mountainous, rural areas and its impact on thyroid functioning among five distinct ethnic communities belonging to two races. The study highlights the importance of region-specific policy formulation for iodine fortification taking into consideration of geographical parameters and ethnicity. To the best of our knowledge, this is the first report on status of soil iodine and its effect on thyroid function in Mongoloid races of India.

MATERIALS AND METHOD

Ethics approval

This study was approved by Institutional Ethics Committee (Bodoland University), Vide letter number IEC/BU/NFST/2020-1 of dated 5th March, 2020.

Consent to participate

Consent was obtained from the participants, guardians of minor prior to the urine sample and blood sample collection.

Sampling methods

Stratified, multiple stage random sampling method was used in the study so that the incidence of disease can represent the whole population. A total of 2142 individuals initially consented to participate in this study however, 142 were excluded since 56 did not complete the questionnaires, 86 did not have blood drawn. 50 numbers of villages predominantly inhabited by the ethnic communities were randomly selected from the whole district. The minimum distance between any two nearest villages were 5km apart. From every village, 40 individuals both male and female and ranging between 10-90 years were selected for UIC, thyroid hormone profiling, anti- TPO Ab.

Inclusion criteria

Patients of the age group, 10-90 years irrespective of their gender were included in the study.

Exclusion criteria

Patients taking thyroid medications, goiter patients, pregnant women, self-reported cases with thyroid disorder and neonates were excluded from the study.

Data collection

This study was carried out during April 2020- March 2021 with the help of Accredited Social Health Activist (ASHA) worker and village head man. Only one individual was included from one household. A standard questionnaire following WHO setup was prepared for recording the data that includes information about medical history, dietary intake and sociodemographic factors.

Collection of soil sample

Two soil samples, topsoil (0-20cm depth) and subsoil (30-50cm depth) from a spot of a kitchen garden of one household from each village were collected. Both the samples were thoroughly mixed and kept in glass containers. The samples were air dried at room temperature and grinded thoroughly to pass a 2mm sieve.

Collection of urine samples

Approximately, 5ml of casual morning spot urine samples were collected in urine cups from 2000 individuals. The samples were kept frozen at -18˚ C until the test is carried out.

Collection of blood samples

Approximately 5 ml of venous blood were collected from every individual under aseptic conditions in a clotted vial with individual ID number. Information of individuals name, sex, age and date of test were recorded.

Soil iodine content

2 gm of air-dried soil was boiled under reflux with 30 ml 2N NaOH for 45 minutes. The mixture was filtered with Whatman no 42-filter paper and centrifuged for 5 minutes at 8000 rpm. Iodine was measured by dry ashing and determined via reduction of yellow- coloured ceric ions to colourless cerous ions by arsenic in presence of iodide [8]. The samples were measured by UV-VIS spectrophotometry.

Urinary iodine content (UIC)-UIC was measured by dry ashing and then its catalytic action on the reduction of ceric ions to cerous ions by arsenic in the presence of

iodide [9]. The decrease in yellow over a given time is measured by colour UV-VIS spectrophotometric method. (SHIMADZU, Model no- UV-19001)

Thyroid Profile Test

The samples were allowed to clot and the serum was separated by centrifugation at 2000 rpm per min for 30 minutes. The serum was labelled and stored in -20 ° C. The thyroid stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3) was measured by chemiluminescence immunoassay method (Roche Diagnostics, Germany). Subclinical hypothyroidism (SCH) was diagnosed with elevated TSH and normal T3 and T4 levels and subclinical hyperthyroidism was diagnosed if TSH was below the normal range and with presence of normal serum T3 and T4 concentrations. Overt hypothyroidism was diagnosed with elevated TSH and low T4 concentration and overt hyperthyroidism by low TSH and high T4 level.

Anti-TPO antibody

Assay of Anti- TPO Ab was performed by electro-chemiluminescence immunoassay analyzer (ECLIA) (Cobas e411, Roche Diagnostics, CA, USA)

Statistical analysis

The data were analyzed by SPSS (Version 26.0., 2019, NY: IBM Corp). Pearson's correlation was used to describe association between the variables. A p value of < 0.05 was considered significant.

RESULTS

Soil iodine content

The average soil iodine content was 2.89 mgkg-1 among the study areas. The highest soil iodine content was with the mean value of 2.41 ± 1.12 mg kg-1 and lowest was with the mean value of 1.07 ± 0.16 mg kg-1.

Urinary iodine content (UIC)

Median urinary iodine (MUI) level was 99.98 µg/l ranging from 18 to 200 µg/l. None of the subjects had UIC ≥300 µg/l. In men, the MUI was 99.89 µg/l and in women 99.99 µg/l. 71.07 % subjects were found to have adequate urine iodine content equal or above 100 µg/l ranging from 100-300 µg/l, 23.91% subjects had mild deficiency which was equal or above 50 µg/l ranging from 50 to 99 µg/l, 5.92 % subjects had moderate deficiency, equal or above 20 µg/l ranging from 20- 49 µg/l (Table 1). Among the euthyroid subjects, 85.92% had adequate urinary iodine, 12.29 % and 1.71 % had mild and moderate urinary iodine deficiency respectively. In hypothyroid subjects, 52.93 % had adequate urinary iodine, 23.15 % and 2.38% had mild and moderate urinary iodine deficiency respectively. In hyperthyroid subjects, 19.66 % had adequate urinary iodine, 1.27 % had mild urinary iodine deficiency. No moderate urinary iodine deficiency was observed among the hyperthyroid subjects (Table 2).

Thyroid Profile Test

Thyroid profile test was based only on the results of biochemical analysis. Out of 2000 individuals, 544 (27.09%) showed abnormal thyroid hormones. 1456 were detected with normal range. Out of 544 subjects, 358 (65.8%) are female and 186 (34.19%) are male which include both hypothyroid and hyperthyroid. Prevalence of hypothyroid is observed in 497 (24.85%) and hyperthyroid in 47 (2.35%) subjects. Among 358 female, 299 (83.51%) females had subclinical hypothyroidism while male had a prevalence of 114 (61.29 %). Overt hypothyroidism in female was 45 (12.56%) and male had 32 (17.20%) subjects. Among hyperthyroid subclinical hyperthyroid was found in 22 (11.82%) males and 7 (1.95%) in female. Overt hyperthyroid in males were 18 (9.67%) and 7 (1.95%) in females (Table 3). The mean (± SD) values of TSH, T3 and T4 are 6.19 ± 4.5, 1.18 ± 0.89 and 6.16 ± 0.91 respectively (Table 4). Among different hypothyroid, subclinical thyroid alteration was seen in 421 subjects (21.05%) and overt hypothyroid in 76 subjects, (3.8%) In hyperthyroid, subclinical alterations were seen in 29 subjects (1.45%) and overt alterations was seen in 18 subjects (0.9%). Subclinical

Table 1: Urinary iodine content values are shown against the ethnicity of the population.

Race	Ethnic groups	Total Subject in tribes	Mean Urinary Iodine (MUI)	% Urine sample ≥100µg/l	% Urine sample ≥50µg/l	% Urine sample ≥200µg/l
Mongoloid	Dimasa	724	99.97	1.2	10.12	1.02
	Hmar	521	96.3	0.9	10.08	2.11
	Kuki	352	98.79	3.51	12.06	1.08
	Naga	301	99.99	5.2	17.05	0.7
Proto Australoid	Tea-Tribe	102	100.1	6.15	10.19	0.1

Table 2: Description of urinary iodine concentration and anti TPO antibody among different conditions of thyroid disorders.

Parameters	Euthyroid	Hypothyroidism		Hypothyroidism	
		Subclinical n (%)	n (%)	Subclinical n (%)	n (%)
Moderate iodine deficiency (µg/l)	26(1.71)	9(1.65)	4(0.73)	0(0)	0(0)
Mild iodine deficiency (µg/l)	179(12.29)	114(20.95)	12(2.20)	5(0.91)	2(0.36)
Adequate iodine (µg/l)	1251(85.92)	207(38.05)	84(14.88)	69(12.68)	38(6.98)
Normal anti- TPO Ab (IU/ml)	1416(96.58)	199(60.30)	51(51)	54(72.97)	30(100)
Elevated anti- TPO Ab (IU/ml)	50 (3.41)	131(39.69)	49(49)	20(27)	0(0)

Parentheses shown in percentage

Table 3: Prevalence of thyroid disorder according to age.

Parameters	Total (n=544)	Hypothyroidism		Hyperthyroidism	
		Subclinical n (%)	Overt n(%)	Subclinical n (%)	Overt n(%)
Female	358 (65.8)	299 (83.51)	45 (12.56)	7 (1.95)	7 (1.95)
Male	186 (34.19)	114 (61.29)	32 (17.20)	22 (11.82)	18 (9.67)
Parentheses shown in percentage					

Table 4: Description of thyroid hormones, urinary iodine and anti TPO antibody.

Parameters	Mean ± SD	<18 Mean ± SD	18-60 years Mean ± SD	>60 years Mean ± SD
TSH (µIU/ml)	6.19 ± 4.5	6.09 ± 0.92	8.18 ± 7.19	6.83 ± 12.11
T_3 (µg/dl)	1.18 ± 0.89	3.31 ± 2.19	1.12 ± 0.04	1.01 ± 0.11
T_4 (ng/ml)	6.16 ± 0.91	6.71 ± 1.81	6.21 ± 0.8	7.26 ± 0.12
Urinary iodine (µg/L)	99.98	99.99	99.86	97.92
Anti TPO antibody (IU/ml)	20.16 ± 39.64	12.21 ± 14.12	42.11 ± 81.12	10.23 ± 13.92
Parentheses shown in percentage				

Table 5: Different types of thyroid disorder according to age group.

Clinical conditions	Overall (n%)	<18 (n%)	18-60 years (n%)	>60 years (n%)
n (%)	544	33 (6.06)	391(71.8)	120(22.05)
Subclinical	421	31 (93.93)	292 (74.68)	98 (4.9)
hypothyroid	76	1 (3.03)	67 (17.13)	8 (6.66)
Overt	29	1 (3.03)	21(5.37)	7(5.83)
hyperthyroid	18	0 (00)	11 (2.81)	7 (5.83)
Parentheses shown in percentage				

hypothyroidism was higher in the age group less than 18 whereas overt hyperthyroid was least in that age group. Overt hypothyroidism, subclinical hyperthyroidism and overt hyperthyroidism were prevalent among 18-60 years of age group (Table 5).

Anti-TPO antibody

12% of the population have anti-TPO Ab positive that is above biological reference intervals (<9IU/mL). Among the euthyroid subjects 3.41% had anti-TPO Ab positivity. Among different types of thyroid disorders subclinical hypothyroidism had the high prevalence of anti-TPO Ab. The percentage of antibody increased along with the age and equally distributed among male and female. The mean (± SD) of anti- TPO antibody is 20.16 ± 39.64 (Tables 2 and 3).

Statistical analysis

Correlation between urinary iodine and anti- TPO antibody: The value of Pearson correlation coefficient between urinary iodine and anti-TPO antibody is -0.07 and P-value is <0.05. The result indicates negative correlation between the variables.

Correlation between age and TSH: The value of Pearson correlation coefficient between Age and TSH is 0.215, which suggest that value of TSH increases with age. The P- value (<0.01) indicates that the relationship between age and TSH is highly significant.

Correlation between age and anti-TPO antibody: The value of Pearson correlation coefficient between age and anti-TPO antibody is 0.139, which suggest positive correlation. The P- value (0.021) indicates that the relationship between age and anti-TPO antibody is significant.

DISCUSSION

From structural as well as functional point of view of thyroid hormone, Iodine is the determinant component. Its concentration must be maintained within the range because both excessive and deficiency have harmful effects. Iodine intake monitoring from dietary source is

important to track the cause of thyroid disease in a population living in goitrogenic belt. Studies on the population's iodine status becomes essential since dietary habits are different in different populations and relevance of such studies are drawn during intervention against IDDs through fortified salts. The prevalence of thyroid abnormalities was more in regions where more than adequate level of iodized salt was used as dietary iodine source [10,11]. On the other hand, low iodine intake is considered as a potential risk factor [12].

In 1980s, a record of severe IDD was reported from eastern province of China due to low soil iodine content [13]. Soils in the high altitude tends to leach away by soil erosion. Low soil iodine content is a concern for goitrogenic belt. Food grown in such soil are iodine deficient. A study conducted among school children in high altitude located Taif city of Saudi Arabia and far away from sea reported high percentage of Goitre [14]. Again, tribal population in coastal regions of Odisha had high numbers of IDD even after ban of sale of non-iodised salt [15]. The Universal standard average iodine content of soil should be 5.1 mg/kg [16]. Our study area showed an inadequate amount of soil iodine with an average of 2.89 mgkg-1 between 1.07 ± 0.16 mgkg-1 and 2.41 ± 0.11 mgkg-1

The WHO/UNICEF/ICCIDD recommended median

value of urinary iodine concentration is 100-200 mg/l [17]. In our study, urinary iodine among these ethnic groups ranged from 18 to 220 µg/l. The percentage of people in the group were differentiated considering the median UIC as 200-300 µg/l, 100-199 µg/l, 50-99 µg/l, 20-49 µg/l, < 20 µg/l indicates above requirements, sufficient, mild, moderate and severe iodine deficiency, respectively based on WHO cut-offs. A survey conducted among school children and adolescents in Uttarakhand

showed adequate iodine status in spite of being situated in hilly region which is contrary to our study [18]. This difference might be due to proper fortification of iodine in food supplies in Uttarakhand. The prevalence of ID in our study is 29.83 % which is very similar to the tribal population of Kashmir (28.4%) [19]. The median urinary iodine in our study population was 134µg/l. 23.91 % subjects had mild urinary deficiency, 5.92 % subjects had moderate urinary. 15.6% female subjects suffered from mild deficiency. The mean urinary iodine in male and female had no distinct difference with the MUI of 139.8 µg/l and 131.1 µg/l respectively. Higher median urinary iodine than adequate is also observed in 5.85% individuals which indicates excessive iodine intake. Excessive intake of iodine increases the risk for iodine-induced hyperthyroidism or hypothyroidism in some vulnerable individuals [20]. In the post- iodization era, the results urge on improvement on iodine status among the ethnic groups. Burden of ID in this tribal population may be due to consumption of non-iodized salt. Due to consumption of non-iodized salt higher prevalence of ID was found among the tribals of Niyamgiri hill range, Orissa (51.7%) and Kerala (87.5%) lying in the coastal regions of India [15, 21].

The prevalence of thyroid disorder in our study population is 27.09 %. Women are seen to have more thyroid disease in our study and is consistent with worldwide reports (66.4% female and 33.57% male). Among different conditions of hypothyroid and hyperthyroid, subclinical hypothyroidism is the most common (21.05% subjects) in our study. 19.3% of subclinical hypothyroidism and 4.2% had overt hypothyroidism in a study conducted in Delhi among non-tribal population which showed lower percentage of subclinical hypothyroidism but similar percentage of overt hypothyroidism (3.8%) in comparison to our study [22]. Among different hypothyroid, subclinical thyroid alteration was seen in 421 subjects (21.05%) and overt hypothyroid in 76 subjects, (3.8%) In hyperthyroid, subclinical alterations were seen in 29 subjects (1.45%) and overt alterations was seen in 18 subjects (0.9%). A study conducted in a health center of mountainous region of Kashmir among women of tribal population showed similar prevalence of subclinical hypothyroidism (21.6%) to our study [23]. In iodine sufficient belt of Cochin, Subclinical hypothyroidism had lower prevalence of about 9.4% which was more common in women than men [21]. A lower prevalence of subclinical hypothyroidism was seen in iodine deficient places of India ranging from 1.8 to 7 %, while higher prevalence was found in iodine sufficient place ranging from 4.9 to 10.4% [24,25]. Tribal women of Godavari district which is iodine sufficient were found to have higher prevalence (36.6%) of subclinical hypothyroidism [26]. The spectrum of thyroid disorder in iodine replete regions were more common in thyroid nodules (7.2%) than hypothyroidism (17.5%) [6]. The higher prevalence of subclinical hypothyroidism than anti TPO antibody representing autoimmune disorder shows iodine deficiency might be a cause for thyroid disorders. The prevalence of overt hypothyroidism (4.9%) was found to be consistent among other studies of India (3.9- 4.2%) [21,22].

The prevalence of hyperthyroidism in this study is little high (2.35%) and to the studies made in Delhi, Cochin with the prevalence ranging from 1.1-1.6% [22].

Anti-thyroid antibodies show progression of subclinical hypothyroidism to overt hypothyroidism in 5-10% of individuals per year and have higher TSH level [23]. In an epidemiological study, 16.7% of the adult subjects had elevated anti TPO antibodies but among them 9.5% of the subjects were without thyroid disorder [5]. 12% of the studied population have elevated anti- TPO antibody where 2.5% of the euthyroid subjects had anti TPO positivity. Increase in anti TPO positivity along with age was seen where 8.1% of the subjects were below 18 years and 19.4% of belong to elderly population. Our result was similar as compared to

other non-tribal states of India [5]. Kerala being an iodine sufficient place, 16.7% showed anti- TPO positivity. Higher incidence of autoantibodies was seen in iodine deficient areas like Brazil and Denmark whereas low incidence was seen in Nigeria which is also deficient in iodine [27,28]. The variation in results may be due to the difference in tribes, genetic susceptibility, lack of international standards and use of different reference interval to define positivity in different studies [19]. Other than environmental and hormonal influences, understanding the molecular basis for thyroid functioning and elucidation of the genetic factors among the ethnic groups will give a concrete idea on how these groups vary in thyroid functioning.

LIMITATIONS

This study did not include the assessment on type and amount of salt consumed by the ethnic population. Other than anti-TPO Ab this study did not assess other anti-thyroid antibodies to give a correct scenario of autoimmune thyroid disorder among the ethnic tribes.

CONCLUSION

The current study showed high magnitude of iodine deficiency among the ethnic population residing in rural areas. The hypothesis that depleted soil iodine content may be the cause of thyroid disorder holds true in our study. The results suggest improvement on iodine status among the ethnic groups of Dima Hasao District. Region-

specific guidelines of iodine intake should be proposed considering the soil health, race and geographical location. The results emphasize a further investigation on cyanogenic food consumption by the ethnic population of the region keeping the environmental determinants in consideration.

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