

To assess the prevalence of Iodine deficiency disorders & deficiency status in selected population by estimation of urinary iodine excretion level.

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Introduction –

Iodine is vital micronutrient used by the body to make thyroid hormones. In body fluids, Iodine is found as iodide (I). In the thyroid gland, with the action of thyroid peroxidase, iodide is oxidised and get incorporated into tyrosine molecule of thyroglobulin (Tg) and form mono - and di-iodotyrosine. These are then coupled to produce Triiodothyronine (T3) and Thyroxine (T4) hormone for standard growth of human and mental development with an average requirement of 100-150 µg/day. Recommended intake of iodine as per UNICEF, ICCIDD and WHO, daily

This study was conducted at the Department of Biochemistry, Index Medical College, Hospital & Research Centre, Indore region (M.P.) between 2020 - 2023.

Method for measuring urinary iodine Using ammonium persulfate.

Principle

Urine is digested with ammonium persulfate. Iodide is the catalyst in the reduction of ceric ammonium sulfate

- 1). Ammonium persulfate (analytical grade)
- 2). As_2O_3
- 3). NaCl
- 4). H_2SO_4
- 5). $\text{Ce}(\text{NH}_4)_4(\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}$

Solutions preparation:

1.0 M Ammonium per sulfate: - Dissolve 14.1 g $\text{H}_2\text{N}_2\text{O}_8\text{S}_2$ in H_2O ; make up to 500 ml with H_2O . Store away from light. The solution is stable for at least one month.

NH_2SO_4 : - Slowly add 139 ml concentrated (36 N) H_2SO_4 , to about 700 ml deionized water (careful- this generates heat!). When cool, adjust with deionized water to a final volume of 1 litre.

Arsenious acid solution: - In a 2000 ml Erlenmeyer flask, place 20 g As_2O_4 , and 50 g NaCl, then slowly add 400 ml 5 N H_2SO_4 . Add water to about 1 litre, heat gently to dissolve, cool to room temperature, dilute with water to 2 litres, filter, and store in a dark bottle away from light at room temperature. The solution is stable for months.

Iodine intake should be as follows: For pre-school children it should be 90 µg; 120 µg for 6 to 12 years; 150 µg/day for adolescents and adults; 250 µg for pregnant and lactating women ^{1,2}.

Thyroid gland may not be able to synthesize adequate amounts of thyroid hormone and this results in hypothyroidism, affects people of both sexes of all ages & of different socioeconomic backgrounds. Due to deficiency of nutritional iodine, the disorders caused are called iodine deficiency disorders (IDDs) ^{3,4,5}.

MATERIAL AND METHOD –

Sample size: -

30 clusters were selected by cluster sampling technique; as per the guidelines from Government of (M.P) ¹⁰³, 70 samples

(yellow) to cerous form (colourless), and is detected by rate of colour disappearance (Sandell - Kolthoff reaction).

Equipment

Heating block (vented fume hood not necessary), colorimeter, thermometer, test tubes (13 x 100 mm), reagent flasks and bottles, pipettes, balance scales.

Reagents

- 6). Deionized H_2O (Method A)
- 7). KIO_3

Ceric ammonium sulfate solution:- Firstly, prepare 3.5N H_2SO_4 - Slowly adding 97 ml concentrated (36 N) H_2SO_4 , to about 800 ml deionized water (careful, this generates heat!) and when cool, adjusting with deionized water to a final volume of 1 litre.

Secondly, prepare Ceric ammonium sulfate solution - Dissolve 48 g ceric ammonium sulfate in 1 liter 3.5 N H_2SO_4 . Store in a dark bottle away from light at room temperature.

Standard iodine solution, (1 mg iodine/ml)

Stock Iodine Standard (1 mg/ml): -168.5 mg KI (potassium iodide) is dissolved in deionized water to make a final volume of 100 ml. This is stored in an amber color bottled, (Weighing for Minimum 0.2 gm: -168.5 mg KI dissolved in 100 ml deionized water)

Dilute Iodine Standard (1µg/ml): Take 100 µl of Stock Iodine Standard and make a volume to 100 ml with deionized water.

Working Iodine Standard: - Make the following serial dilutions from diluted Iodine Standard (1 µg/ml) into volumetric flasks (10 ml) with deionized water. (Diluent). These dilutions are made freshly.

Procedure

1. Mix urine to suspend sediment.
2. Pipette 250 l of each urine sample into a 13 x 100 mm test tube.
Pipette each iodine standard into a test tube, and then add H₂O as needed to make a final volume of 250 l. Duplicate iodine standards and a set of internal urine standards should be included in each assay.
3. Add 1 ml 1.0 M ammonium per sulfate to each tube.
4. Heat all tubes for 60 minutes at 100 °C.
5. Cool tubes to room temperature.
6. Add 2.5 ml arsenious acid solution. Mix by inversion or vortex. Let stand for 15 minutes.

7. Add 300 µl of ceric ammonium sulfate solution to each tube (quickly mixing) at 15 to 30-second intervals between successive tubes. A stopwatch should be used for this, with practice; a 15-second interval is convenient.

8. Allow to sit at room temperature. Exactly 30 minutes after the addition of ceric ammonium sulfate to the first tube, read its absorbance at 405 nm. Read successive tubes at the same interval as when adding the ceric ammonium sulfate.

Inclusion criteria:

- Age between 6-12 years
- Rural area of Indore District

Exclusion Criteria:

- All those children below 6 years or above 12 years as per the records in the school were excluded from the study.
- Patients with diagnosed thyroid disease and/or other chronic diseases;
- Any significant background illness;
Exposure to any iodine containing medications except multivitamin and mineral supplements.

RESULTS –

Table – 1. Distribution of Urinary Iodine (n= 2100).

Distribution of Urinary Iodine (n = 2100)		
Urinary Iodine (µg/L)	n	%
< 20	0	0
20-49	5	0.23
50-99	56	2.66
100-199	1883	89.66
≥ 200	156	7.4

Graph-1: Urinary Iodine (µg/L).

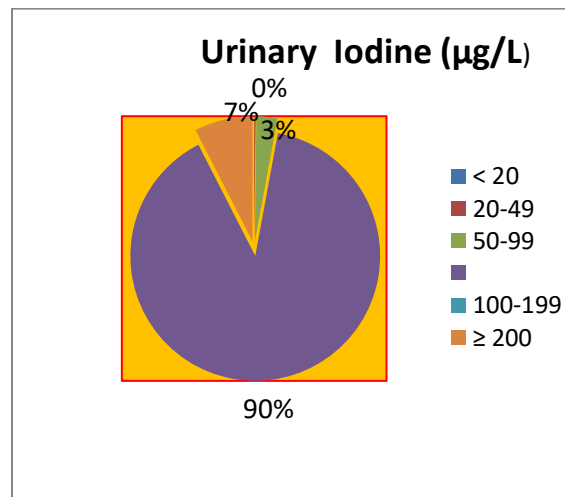


Table- 2: Age-wise Distribution of Urinary Iodine (n =2100).

Age-wise Distribution of Urinary Iodine (n=2100)											
Age (Yrs)	Total n	< 20 µg/L		20-49 µg/L		50-99 µg/L		100-199 µg/L		>200µg/L	
		n	%	n	%	n	%	n	%	n	%
6	172	0	0	0	0	4	2.32	160	93.02	14	8.1
7	302	0	0	1	0.33	4	1.32	262	86.75	24	7.9
8	294	0	0	2	0.68	5	1.7	274	93.19	25	8.5
9	305	0	0	0	0	5	1.63	263	86.22	33	10.81
10	309	0	0	0	0	5	1.61	280	90.61	20	6.47
11	408	0	0	2	0.49	18	4.41	373	91.42	19	4.65
12	310	0	0	0	0	15	4.83	269	86.77	23	7.4

Table-3: Age-wise Distribution of Urinary Iodine (n=2100).

Age (Yrs)	Urinary Iodine excretion				
	Total n	n	>100µg/L(%)	n	< 100 µg/L(%)
6	172	171	(99.41)	1	0.58
7	302	288	95.36	14	4.6
8	294	292	99.31	2	0.68
9	305	297	97.37	8	2.6
10	309	296	95.79	13	4.2
11	408	394	96.56	14	3.4
12	310	292	94.19	18	5.8
Total	2100	2030	96.66	70	3.33

Table-4: Age wise analysis of Urinary Iodine excretion.

Age (Yrs)	Urinary Iodine excretion				
	Total n	n	>50µg/L (%)	n	<50 µg/L(%)
6	172	172	0	0	0
7	302	300	99.33	2	0.66
8	294	291	98.97	3	1.02
9	305	305	0	0	0
10	309	309	0	0	0
11	408	405	99.26	3	0.73
12	310	310	0	0	0
	2100	2092	99.61	8	0.38

Table - 5: Gender- wise Distribution of Urinary Iodine (n- 2100).

Gender-wise Distribution of Urinary Iodine (n=2100)											
Gender	n	< 20 µg/L		20 -49µg/L		50-99 µg/L		100-199 µg/L		>200 µg/L	
		n	%	n	%	n	%	n	%	n	%
M	1050	0	0	3	0.28	31	2.95	934	88.95	82	7.80
F	1050	0	0	3	0.28	24	2.28	949	90.38	74	7.04

Graph-2: Urinary Iodine Excretion (UIC) level in Indore district (µg/L).
UIC (g/L)

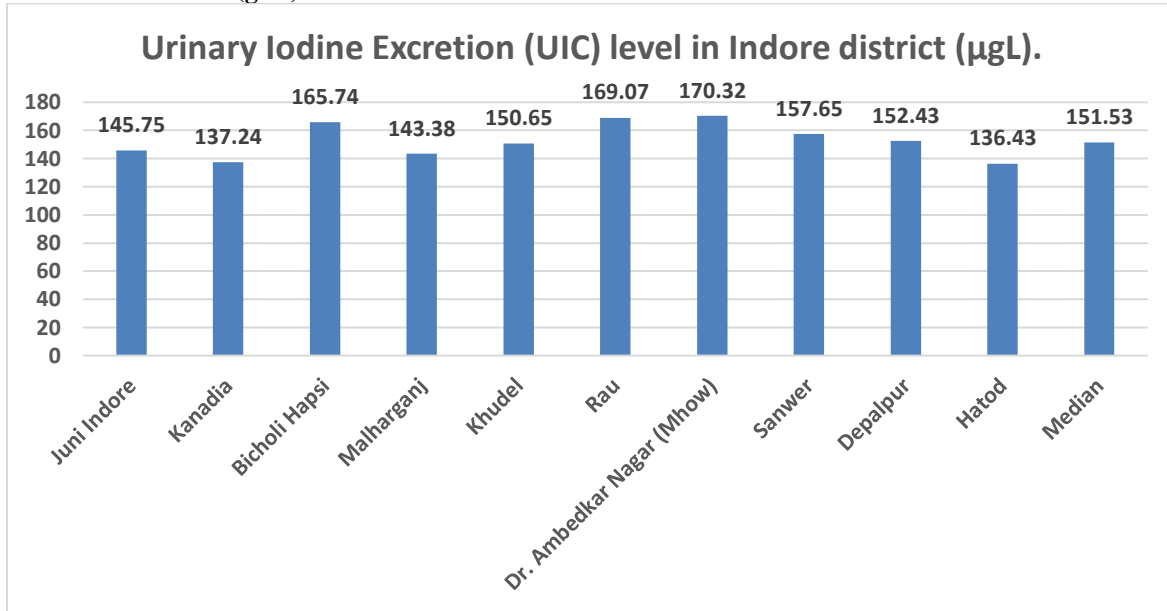


Table-6: Urinary iodine excretion level in rural areas of Indore district

Tehsil/Taluka	Children examined	Urinary Iodine Excretion(UIE)	
		>50 µg/L(%)	<50 µg/L(%)
Juni Indore	210	209 (99.52)	1 {0.47}
Kanadia	210	210 (100)	0 (0)
BicholiHapsi	210	208 (99.04)	2(0.95)
Malharganj	210	209 (99.52)	1 {0.47}
Khudel	210	210 (100)	0 (0)
Rau	210	210 (100)	0 (0)
Dr. Ambedkar Nagar (Mhow)	210	210 (100)	0 (0)
Sanwer	210	210 (100)	0 (0)
Depalpur	210	209 (99.52)	1(0.47)
Hatod	210	210 (100)	0 (0)
Total	2100	2095(99.76)	5 (0.23)

Table-7: Urinary iodine excretion level in rural areas of Indore district.

Tehsil/Taluka	Children examined	Urinary Iodine excretion(UIE)	
		>100 µg/L(%)	<100 µg/L(%)
Juni Indore	210	210 (100)	0 (0)
Kanadia	210	198 (94.28)	12 (5.7)
BicholiHapsi	210	210 (100)	0 (0)
Malharganj	210	200 (95.23)	10(4.7)
Khudel	210	210 (100)	0 (0)
Rau	210	205 (97.61)	5(2.3)
Dr. Ambedkar Nagar (Mhow)	210	207 (98.57)	3 (1.4)
Sanwer	210	196 (93.33)	14 (6.6)
Depalpur	210	198 (94.28)	12 (5.7)
Hatod	210	198 (94.28)	12(5.7)
Total	2100	2032 (96.76)	68(3.23)

DISCUSSION

The knowledge about the necessity of vitamin B12 supplementation is well-known among VN, and the prevalence of vitamin B12 deficiency is low as shown in a previous study⁶ or Vechi Youth study⁷. On the other hand, we lack information about their iodine supplementation habits, nutritional intake, and iodine saturation. Therefore, we conducted this study to describe iodine saturation, intake, and supplement use in Czech children consuming plant-based diets and to assess whether these children are at possible risk of iodine deficiency, as reported in cross-sectional studies in adults. The study was designed as a cross-sectional study, and we examined a total of 221 children from November 2019 to July 2021 in our facility in the Indore region. Although the Czech Republic is considered a country that has solved iodine deficiency as a public health issue since 2004, we may have identified a new population at risk, namely children following a VN and VG diet. Unlike pregnant and lactating women, public health authorities have not paid attention to this group so far.

We observed that

(1) The mean daily intake of iodine is significantly lower among VN compared to OM and VG children from the Czech Republic.

The following points have been concluded in the present studies, which are mentioned below:

1 - Construct a standard curve on graph paper by plotting iodine concentration of each standard on the abscissa against its optical density at 405 µg/l (OD₄₀₅) on the ordinate. For each unknown specimen, find its log (Abs) on the standard curve and read off the corresponding iodine concentration (µ /L). Any specimens that have log (Abs) values lower than the standard curve should be

(2) These differences were mirrored by a lower median UIC among VN children compared to OM children but all of them were >100 µg/l.

(3) There were statistical significant differences in levels of fT4 between the groups, but no differences in TSH, fT3 and TG.

(4) The VG/VN group exhibited a higher prevalence of AhTGc positivity; the difference in ATPOc was not statistically significant. We observed the highest iodine intake among the VG group, lowest in the VN group, with intermediate intake levels in the OM control group.

This is in partial agreement with other published studies where VN tend to have the lowest^{8,9} intake, although some studies show the highest intake of iodine compared to other groups^{10,11} possibly due to excessive seaweed consumption^{10,11,12}. OM has the highest estimated average intake of iodine in most studies¹³. In one previous study among children, iodine intake was lowest in the VN group and highest in OM group, with intermediate intake levels among VG children⁷. VG children may have the highest intake because they tend to substitute meat with dairy and eggs that are the main source of iodine in diet when compared to meat in

Conclusion -

diluted (1 part urine to 1 part deionised water) and re-assayed.

2-The median urinary iodine concentration found is 151.45 µg/L which is 89.57% found in the optimal range which indicate the proper iodine intake.

3- 91.66% of salt samples had iodine levels > 15 ppm and the iodine content in salt samples < 15 ppm was 8.33% indicating the salt samples at household contain sufficient levels of iodine as per criteria.

4- 0.61% goitre cases have been reported i.e. 13 out of 2100 samples. 9 cases were from grade-1 and 4 cases were

from grade-2. Maximum prevalence rate was observed in the age group of 11 year children i.e. 0.98% in which two cases was from grade-1 and two cases were from grade-2. 0.76% prevalence rate was observed in female children and 0.47% prevalence rate was observed in male children out of total sample.

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