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A Study of Urinary Excretion of Iodine in Malnourished (Children)

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Abstract: ***Aim:-To study the Urinary excretion of Iodine in malnourished (children) subjects. Methodology: Study design-Study to carry out IDD in hospital attended (children) subjects due to different nutritional and non-nutritional disorders. Results: Results of the urinary excretion of iodine are given in table 11. No clinical evidence of goiter was detected in the study groups specially which was examined in age 3-12 years. Urinary iodine varied from 2.5-31-µg/ml. Mean ± SD of urinary iodine excretion was found to be 12.8 ± 9.2 µg/ dl. The prevalence of median value was found to be 10 µg/dl. The prevalence of different grades of IDD is given in table (11). According to ICCIDD classifications, 58.8 of the subjects were found to be normal (urine iodine excretion more than 10 µg/dl). Mild IDD were seen in 23.5 % of the subjects (whose urinary iodine excretion was 5-9.9 µg/dl). Moderate IDD were observed in 17.6% (urinary iodine excretion was 2-4.9µg/dl). The incidence of severe forms of IDD in (less than 2µg/dl) the subjects studied was nil. Discussion: Though various methods of assessing iodine deficiency are available today, we applied both biochemical and clinical evaluation of the subjects. There was no evidence of severe IDD in the study group. The statistical analysis revealed only mild to moderate degree of IDD. Median urinary iodine excretion was 10µg/dl which was less than that reported by Pandav et al (1994) in Delhi School children. He reported a value of 19.8 µg/dl, which was more than the mean urinary iodine of 6.5 µg/dl and 4.8µg/dl reported in 1981 and 1979, respectively. However, he reported that 24.9% of School children had urinary iodine les than 10 µg/dl. In this study we found that 41.1% of malnourished children had urinary iodine less than 10µg/dl. This indicates that through their daily diet. It is suggested that strengthening of IDD programmes is the only solution to reduce the prevalence rate.***

Keywords: Iodine deficiency, Malnourished, urinary excretion

1. Introduction

Iodine deficiency disorder (IDD) is a global public health problem. No country is yet been recorded to over come it. Though various methods are available to find out IDD, urinary (random) iodine excretion is one of the very cost effective, easy as well as most sensitive indicator of iodine metabolism.

1.1 Principles of the Test

Urine is the digested with chloric acid under mild condition and iodine is determined manually by its catalytic role in the reduction of ceric ammonium sulfate in the presence of arsenic acid. As the reduction precedes the intensity of color decreases and this can be readily measured in the spectrophotometer at 420 nm. The method is fast and inexpensive and the digestion is les harsh than some other methods. This method can measure urinary iodine concentration in the range of 0-150 µg/L but can be extended further to cover a wider range of values.

1.2 Equipments and Chemicals

1.2.1 Equipments

Oven with fan exhaust, vented fume hood an oven for perchloric acid escape, UV spectrophotometer, thermometer, timer (stop watch reliable to 5 seconds, test tubes (15 mm X 100 mm), funnel (36X 100mm) reagents flasks and bottles, pipettes, Whitman's no. 1 filter paper and a laboratory balance.

1.2.2 Chemicals (analytical grade AR/GR)

i) KCL_3 (potassium chlorate)
ii) $HCLO_4$ (perchloric acid, 70%)
iii) AS_2O_3 (arsenic trioxide)
iv) NaOH (sodium hydroxide)
v) H_2SO_4 (sulfuric acid)
vi) $Ce(NH_4)_4(SO_4)_4 2H_2O$ (ceric ammonium sulfate)
vii) KIO_3 (potassium iodate)
viii) HCL (Hydrochloric acid)
ix) Double distilled water (free of iodine and other contaminants)

1.3 Preparation of reagents

1) **Choric acid solution:**
IN A 2000 ML Erlenmeyer flask, 500g potassium chlorate was dissolved in 190 ml hot double distilled water until the soluble state (normally a little amount remains undissolved). 375 ml of 70% percloric acid was added drop wise (approx.15 ml/min) while stirring constantly. This preparation was carried out in a vented fume hood as it produces toxic fumes. Subsequently, the solution was kept in a freezer of refrigerator overnight for better separation. The next day it was filtered through a filter paper, (Whatman's No.1) and stored in a refrigerator at 4^{O} C.

2) **Arsenic Acid Solution:**
0.986 g arsenic trioxide was taken in a 1000 ml volumetric flask and was dissolved in a 10 ml of 0.5 N hot sodium hydroxide. This solution was transferred into 750 ml chilled double distilled water. Than 20 ml concentrated HCL and 39.6 ml conc. Sulfuric acid

(98%) was added drop wise with constant mixing. The solution was stored in amber color bottle at room temperature. (The solution is stable for months).

3) **Sulfuric Acid Solution (3.5 N H_2SO_4):**
97 ml concentrated sulfuric acid (98%) was added drop wise into 800 ml chilled double distilled water (carefully as this generates heat) and final volume was made up to 1 liter with double distilled water.

4) **Ceric ammonium sulfate solution:**
48 g ceric ammonium sulfate was dissolved in 1 liter of 3.5N H_2SO_4. This was stored in a amber color bottle at room temperature (the solution is stable for months).

5) **Stock Iodine Standard (1mg/ml):**
168.5 mg KIO_3 was dissolved in double distilled water to make a final bottle (this solution is stable for months).

6) Dilute Iodine Standard (1u/ml): Take 100 µl of stock iodine standard and make a volume to 100 ml with double distilled water (once a week).

7) Working Iodine Standard: Make the following serial dilution from dilute Iodine Standard (1 µg/ml) into volumetric flask (10ml) with double distilled water. These dilutions are made freshly (fresh solutions).

µg/ml	Dilution factors
5µg :	0.5 ml of 1 µg/ml standard + 9.5 ml diluents
10µg :	1.0 ml of 1 µg/ml standard + 9.0 ml diluents
5µg :	1.5 ml of 1 µg/ml standard + 8.5 ml diluents
20µg :	2.0 ml of 1 µg/ml standard + 8.0 ml diluents.

2. Procedure

Step 1: the urine sample was shaken to evenly suspend any sediment. 250 ml of each urine sample was pipetted into a 15x 100 ml test tube. Iodine standards were prepared from the 1µg /ml stock iodine solution. The iodine standards corresponding to 0/5/10/15 and 20 µg/ml were prepared.

Step 2: 750 µg/ml of chloric acid solution was added to each tube (samples, blanks, internal quality control sample, standards) and mixed gently. All tubes were placed in the oven at 110 ^{0} C- 120 ^{0} C for 75 minutes (with a fume hood for the trapping of perchloric acid). There will be very little volume change during heating; some samples may be faintly yellow. All the tubes were cooled at room temperature for 15 minutes. Then, the decreased volume was adjusted with double distilled water to their original volume (1.0) ml and vortexed.

Step 3: 3.5 ml of Arsenic Acid was added to each test tube and after mixing all test tubes were kept for 15 minutes at room temperature.

Step 4: 350 µl of ceric ammonium sulfate solution was added at a fixed interval of time to each tube and quickly mixed with the help of a vortex. A stopwatch was used to keep a constant interval between additions to successive tubes. (30 seconds was a conventional interval). Exactly 20 minutes or a pre-determined time after addition of ceric ammonium sulfate to the first tube, the reduction was read spectrophotometrically at 420 nm. (Successive tubes were arranged in such a manner that the interval between the times of addition of ceric ammonium sulfates and the time of the reading was the exactly 30 sec. For all samples, standards and blanks.

1.5 Calculations of results: the exact value of urine samples iodine was calculated as follows:

1) The average absorbance value for each set of reference standard, control and samples was calculated.
2) A standard curve was constructed by plotting mean absorbance obtained for each reference standard against its concentration ug/ml on linear
3) graph paper, with absorbance on the vertical (Y) axis and concentration (micro µl/dl ml) on the horizontal (X) axis.

3. Precautions

1) Since the digestion procedure has no specific end point, it is essential to run blanks and Iodine standards with each assay to allow for variation in heating time etc.
2) The exact temperature, heating time and cooling time can vary. However, within each assay, the interval between the time of addition of ceric ammonium sulfate and the time of reading must be the same for all samples, standards and blanks.
3) In this procedure it is convenient to run 60 samples tube per assay of which 5 are standards (at concentration of 0/5/10/15 and 20 µl/dl).
4) Perchloric acid fumes can be toxic and the complex generated may be harmful, particularly if allowed to dry in a ventilation system. The recommended method releases much less perchloric acid than other digestion methods.
5) The time and temperature is not critical as long as all tubes are heated the same day.
6) 1.68 mg $KI0_3$ contains 1 mg iodine. $KI0_3$ is prepared over KI because it is more stable.
7) Test tubes can be reused if they are carefully washed to eliminate any iodine concentration.
8) Separation pipettes should be used all the test tubes and also pipettes used for preparation of each standard solution should be kept separately and not be mixed with the general pool of glasswares. They should be kept separately for all times to avoid contamination.

4. Results

Results of the urinary excretion of iodine are given in table 11. No clinical evidence of goiter was detected in the study groups specially which was examined in age 3-12 years.

Urinary iodine varied from 2.5-31-µg/ml. Mean ± SD of urinary iodine excretion was found to be 12.8 ± 9.2 µg/ dl. The prevalence of median value was found to be 10 µg/dl. The prevalence of different grades of IDD is given in table (11). According to ICCIDD classifications, 58.8 of the subjects were found to be normal (urine iodine excretion more than 10 µg/dl). Mild IDD were seen in 23.5 % of the subjects (whose urinary iodine excretion was 5-9.9 µg/dl). Moderate IDD were observed in 17.6% (urinary iodine

excretion was 2-4.9µg/dl). The incidence of severe forms of IDD in (less than 2µg/dl) the subjects studied was nil.

5. Discussion

Though various methods of assessing iodine deficiency are available today, we applied both biochemical and clinical evaluation of the subjects. There was no evidence of severe IDD in the study group. The statistical analysis revealed only mild to moderate degree of IDD.

Median urinary iodine excretion was 10µg/dl which was less than that reported by Pandav et al (1994) in Delhi School children. He reported a value of 19.8 µg/dl, which was more than the mean urinary iodine of 6.5 µg/dl and 4.8µg/dl reported in 1981 and 1979, respectively. However, he reported that 24.9% of School children had urinary iodine les than 10 µg/dl. In this study we found that 41.1% of malnourished children had urinary iodine less than 10µg/dl. This indicates that through their daily diet. It is suggested that strengthening of IDD programmes is the only solution to reduce the prevalence rate.

Conflict of Interest

None

Source of funding

Self

Declaration of Authors

We declared that authors named in the article did this all ground level research including samples collection to test results.

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Sl. No	Tube/sample	Volume Sample (µl)	Volume of water (µl)	Chloric acid (AS_2O_3)	Ceric ammonium Sulphate (µl)	O.D at 420 nm
	Standard					
1	Blank	-	250	750	3.5 ml	0
2	5µl/dl	250	-	750	,,	0.356
3.	10µl/dl	250	-	750	,,	0.331
4	15µl/dl	250	-	750	,,	0.284
5	20µl/dl	250	-	750	,,	0.246
6	30µl/dl	250	-	750	,,	0.156
	Random samples					
1						0.272
2						0.340
3						0.328
4						0.330
5						0.291
6						0.330
7						0.296
8						0.320
9						0.373
10						0.337
11						0.360
12						0.195

S. No	Tube/sample	Volume Sample (µl)	Volume of water (µl)	Chloric acid (AS_2O_3)	Ceric ammonium Sulphate (µl)	O.D at 420 nm
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6			0.330
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8			0.320
9			0.373
10			0.337
11			0.360
12			0.195

Table 2

Iodine level	No. of Subjects	% 0f the subjects\
>2µl/dl	Nil	0
<5µl/dl	3	17.6
5-10µl/dl	4	23.5
>10µl/dl	10	58.8

Table 3: Classification of IDD According to ICCIDD

S.No	Grades of IDD	Level of Urinary iodine
1	Severe	>2µl/dl
2	Moderate	2-4.9µl/dl
3	Mild	< 5-10µl/dl
4	Normal	<10 µl/dl

Table 4

S.No	Patients	Age	Sex	Anemia	Jaundice	Hairs	Skin	Thyroid	Random urinary iodine level µl/dl
1	Kusuma	3yrs	F	Mild	Nil	Sparce	Wrinkling	N/P	17.0
2	Naglakshmi	7yrs	F	Mild	Nil	Normal	Normal	Do	7.0
3	Jayanti	8 yrs	F	Nil	Nil	Normal	Normal	Do	10.0
4	Prasanti	12yrs	F	Nil	Do	Do	Normal	Do	10.0
5	Hemlatha	10yrs	F	Mild	Do	-	Sparce	-	14.0
6	Shanti	12yrs	F	Mild	-	-	-	-	10.0
7	Bhubaneswari	8 yrs	F	-	Do	-	-	-	12.5
8	Md.Hazi	10 yrs	M	-	-	-	-	-	2.5
9	Md.Efran	4 yrs	M	-	-	-	-	-	2.5
10	Sundarya	7 yrs	F	-	-	-	-	-	7.0
11	Aruna	11yrs	F	Mild	-	-	-	-	4.0
12	Nagarjuna	9 yrs	F	Nil	-	-	-	-	26.5
	Adults								
1	Mr.k.Raman	58yrs	M	Mild	-	-	-	-	21.0
2	Mr.M.Ramulu	36 yrs	M	Nil	-	-	-	-	31.0
3	Mr.Shanker Goud	35yrs	M	Nil	-	-	-	-	6.0
4	Mr.Sheik Rasiuddin	18 yrs	M	Nil	-	-	-	-	5.0
5	Mr.L.V.N Reddy	45 yrs	M	-	-	-	-	-	31.0
							Mean Median value		12.8 ±9.2 10.0µl/dl

Reagents For Urinary Iodine

1. Chloric Acid Solution:

25 g of KIO_3 Potassium chlorate oxygen mixture +91 ml double distilled water.

↓

Mild heat with continuous stirring

↓

Completely dissolved and add another 25g of KIO_3

↓

Heat till clear solution obtained (normally 100% clear solution is not obtained. Some $KCLO_3$ don't dissolve till end).

↓

Add 37.5 ml perchloric acid. Keep overnight in freezer of refrigerator.

2. Arsenic Acid

0.20 g NaoH + 0.986 g of AS_2O_3 + 10 ml double distilled water.
↓
Heat mildly till completely dissolved.
H_2SO_4
Make up volume to 750 ml with double distilled water
↓
20 ml Conc. Hcl + 39.6 ml Conc. H_2SO_4
↓
Make volume to 1000 ml with double distilled water (keep at room temperature).

3. Ceric Ammonium Sulfate Solution:

4.8 g of ceric ammonium sulfate + 90 ml distilled water.
Add 9.7 ml Conc. H_2SO_4
↓
Make up volume to 100 ml with double distilled water (keep at room temperature).

4. Stock Iodine Solution: (1µl/ml): 168.5 mg KIO_3 was dissolved in double distilled water to make a final volume of 100 ml. This was stored in an amber color bottled (This solution is stable for months).

Iodine Free Glassware

Preparation of Chromic Acid:

Saturated solution of $K_2Cr_20_7$ (28g) was prepared in 500 ml of distilled water. Add 500 ml of Technical / Commercial grade conc. H_2SO_4 slowly (solution of potassium dichromate is in chilled condition, heat evolved will be less). Discard chromic acid if turned green / light green.

1) Dip the glassware for 24 hours in chromic acid.
2) Use gloves to take out glassware from chromic acid and than wash repeatedly with tap water. Rinse twice with distilled water and finally double water.
3) Dry at 80-100^0 C oven

Pooled Sample

Once the method is standardized, prepared the urine for internal quality assessment (Use pooled sample, mixed properly or collect from one individual about 250-300ml). Analyze the sample 20-25 times with standard and blanks in duplicate. Calculate mean and standard deviation. The value of this sample or dilute the sample should be between 5-15 µl/dl. If not collect fresh sample or dilute the sample with double distilled water to get proper range. Store, this sample in small aliquots of approximately 1.0 ml (100 to 150 aliquots) in refrigerator (4-8^{0}C) and analyze one aliquot with every batch of unknown samples.

For analysis of the unknown samples, use the batch of 40 tubes (Two tubes for blank 4 standards (5, 10, 15 20 µl/dl) , one internal quality assessment sample and once in three weeks one external quality assessment sample + remaining unknown samples).

In case the internal quality assessment sample shows value above + 2 SD or below – 2 SD. The whole batch of unknown sample to be repeated. Check the possible error (Contamination, not proper washing, reagent quality etc) before reusing another aliquots of another internal quality assessment.