METHOD AND MATERIALS

The entire state of Uttarakhand in India is a known iodine endemic state for more than 6 decades. A significant progress has been made in the control of Iodine Deficiency Disorder through supply of Iodised salt. The pregnant mothers, neonates, school age children (6-12 years) and adolescent girls are the most vulnerable groups as they are especially sensitive to even marginal iodine deficiency. There is lack of data available on affects of iodine deficiency on these 4 vulnerable groups from Uttarakhand state. Hence, the study was conducted to assess the iodine status amongst pregnant mothers, neonates, school age children (6-12 years) and adolescent girls in three regions of Uttarakhand state, utilizing indicators recommended by WHO, 2007. Moreover, we do not have data on the environmental influences (iodine content in water and food samples) on iodine deficiency from Uttarakhand state.

Thus to fill the gap in the existing knowledge the present study was conducted with the following objectives:

Objectives of the Study:

1. Assessment of iodine nutritional status amongst pregnant mothers, neonates, school age children (6-12 years) and adolescent girls in the selected 3 districts namely Nainital, Udham Singh Nagar and Pauri Garhwal, in three regions of Uttarakhand namely: i) Kumaon, ii) Terain (Plain) and iii) Garhwal.

2. Assessment of Iodine content of salt consumed by the population in the selected 3 districts namely Nainital, Udham Singh Nagar and Pauri Garhwal, in three regions of Uttarakhand namely: i) Kumaon, ii) Terain (Plain) and iii) Garhwal.

3. To assess Environmental influences; iodine content in water and food samples of Uttarakhand state.
Different stakeholders that were involved in the study were:

1. Department of Foods and Nutrition, Faculty of Family and Community Sciences, The Maharaja Sayajirao University of Baroda, Gujarat
2. All India Institute of Medical Sciences, New Delhi
3. Department of Health, Government of Uttarakhand
5. Department of Women and Child development, Government of Uttarakhand
6. District Magistrates, Government of Uttarakhand
7. National Institute of Nutrition, Hyderabad
8. Lifecell Laboratory, New Delhi

Permissions Obtained

1. The administrative permissions were obtained from the Chief Secretary, Government of Uttarakhand.

2. The administrative permissions were also obtained from District Magistrate, Chief Education Officer, Chief Medical Officer, Medical Superintendent, Gynecologist and Pediatricians of the respective hospitals of three districts of Uttarakhand state.

Ethical Approval

Ethical approval was taken from Ethics Committee of Department of Foods and Nutrition, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat and All India Institute of Medical Sciences, New Delhi.

Funding

This was an Indian Council of Medical Research (ICMR) funded project and Ms. Neha Sareen (Ph.D Scholar) worked as a Lead Research Scientist who was involved in development of the proposal, data collection, implementation of the study, data entry, data analysis, report writing and research publications.
Since, there were four vulnerable groups and environmental influences thus the study was conducted in five parts. They are as follows:

**Part 1:** Iodine Nutritional status amongst Pregnant Mothers

**Part 2:** Iodine Nutritional status amongst Neonates

**Part 3:** Iodine Nutritional status amongst School age Children (6-12 years)

**Part 4:** Iodine Nutritional status amongst Adolescent Girls (12-18 years)

**Part 5:** Environmental Factors (Iodine Content in Water and Food samples)

**Study Area**

The state of Uttarakhand has 13 districts (Dehradun, Uttarkashi, Chamoli, Dehradun, Pauri Garhwal, Tehri Garhwal, Rudraprayag, Haridwar, Almora, Pithoragarh, Nainital, Bageshwar, Champawat and Udham Singh Nagar) which are distributed in three 3 administrative and geographical regions namely i) Kumaon ii) Terai (Plain) and iii) Garhwal. One district was selected from each region i.e. Nainital, Udham Singh Nagar and Pauri Garhwal (Figure 17).
Study population

In the selected district, the status of ID was assessed in the following vulnerable age groups:-

- Pregnant mothers
- Neonates
- Children in the age group of 6-12 years
- Adolescent Girls
Sampling Technique

In the districts selected, 30 clusters were identified by utilizing population proportionate to size sampling (PPS) methodology recommended by WHO for IDD survey. In each clusters, subjects (school age children, adolescent girls, pregnant mothers and neonates) were covered. The children and adolescent girls were covered by school based approach as more than 95% children attended the schools. While the pregnant mothers were covered from Anganwadi centers and the neonates were covered from hospitals.

The figure 18, 19, and 20 indicate the distribution of clusters included in each of district surveyed: Nainital, Udham Singh Nagar and Pauri Garhwal.
Figure 18: Clusters selected in district Udham Singh Nagar
Figure 19: Clusters selected in district Nainital
Figure 20: Clusters selected in district Pauri Garhwal
3.1 Part 1: Iodine Nutritional status amongst Pregnant Mothers

Study Population: Pregnant Mothers attending antenatal clinics

Study Area: Three districts of Uttarakhand namely: Udham Singh Nagar, Nainital and Pauri Garhwal.

Sampling Technique: The study participants were selected by adopting two stage sampling technique. In each district, first 30 clusters (Villages) were identified by utilizing PPS methodology as recommended by WHO/UNICEF/ICCIDD (2007). In the second stage, in each cluster (village), sixteen PMs who were attending the antenatal clinics were included.

Clinical Examination

Prior training for clinical examination was imparted to all the research team members at All India Institute of Medical Sciences, New Delhi. The clinical examination of thyroid of each Pregnant Mother was conducted. The clinical examination of thyroid gland was conducted by Palpation Method and graded as recommended by WHO (2007). The grading of goitre was done according to the criteria recommended jointly by WHO/UNICEF/ICCIDD (a) Grade 0- not palpable and not visible; b) Grade I- palpable but not visible; and c) Grade II- palpable and visible). When in doubt, the immediate lower grade was recorded. The intra- and inter-observer variation was controlled by repeated training and random examinations of goitre grades. The sum of Grade I and II provided the Total Goitre rate (TGR) in the study population.

Biochemical Parameter

In each cluster (village), 13 casual urine samples were collected from the list of pregnant mothers by the research team members and they were enrolled for clinical thyroid examination. Plastic bottles with screw caps were provided to each pregnant mother for the urine samples. The samples were stored in the refrigerator until analysis. The analysis was done within 2 months. The UIC levels were analyzed at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi. The UIC levels were estimated using the wet digestion method (Dunn et al, 1993).
Iodine content in the salt

From each cluster (village) a minimum of 11 pregnant mothers were selected and were provided with an auto seal polythene pouches with an identification slip. They were requested to bring four teaspoons of salt (about 20g) from their family kitchen and the iodine content of the salt samples was analyzed at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi. The iodine content of salt was estimated by standard iodometric titration method (Karmarkar et al, 1986).

Since iodine crystals are smaller than salt crystals and hence they get settled at the bottom of the pack. Thus, pregnant mothers were instructed to mix the sample of salt properly before sampling.

Inclusion and Exclusion Criteria

Inclusion criteria:

a) Subjects who have given the oral consent

Exclusion criteria:

a) Pregnant women consuming drugs which can influence their thyroid status
b) Pregnant women who had multiple pregnancy

Sample Size: The sample size of 450 pregnant mothers was estimated keeping in view of prevalence of iodine deficiency as 5%, confidential interval of 95% and relative precision of 3% and design effect of 2. However, we included 632 (Udham Singh Nagar), 614 (Nainital), and 481 (Pauri) of Pregnant Mothers.
FLOW CHART
Uttarakhand State

3 regions

Terai  Kumaon  Garhwal

3 districts

Udham Singh Nagar  Nainital  Pauri Garhwal

using PPS Methodology

30 Clusters (Villages) from each district

Antenatal clinics organized in 30 clusters were identified

Pregnant mothers were selected from these Antenatal clinics

Clinical Examination  Biochemical Parameter  Iodine content in salt
(n=16/cluster)  (n=13/cluster)  (n=11/cluster)

Goiter  Urinary Iodine Concentration  Iodine Content in salt
(By Palpation Method)  (By Wet Digestion Method)  (By Titration Method)
3.2 Part 2: Iodine Nutritional status amongst Neonates

Study Population: Neonates

Study Area: Three Districts of Uttarakhand namely: Udham Singh Nagar, Nainital and Pauri Garhwal.

Sampling Technique: In the districts selected, 30 clusters (Villages) were identified by utilizing population proportionate to size sampling (PPS) methodology recommended by WHO (2007) for IDD survey. In the identified 30 clusters (villages), all the Hospitals/CHCs/PHC/Institutions catering to the population for obstetric services were identified and enlisted. A total of 6 Hospitals/CHCs/PHC/ Institutions were selected. The 450 births occurring in these Hospitals/CHCs/PHC/ Institutions during the 12 months of study period were included for estimation of neonatal Hypothyroidism. The umbilical cord blood sample were taken on a filter paper and transported to the Central Laboratory in Delhi for estimation of Thyroid Stimulating Hormone (TSH). The service of a Central Laboratory which was accredited by National Accredited Biological Laboratory (NABL) was utilized for estimation of TSH.

Nutrition education Pamphlets was developed by the research scholar along with other research team members at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi for cord blood sample collection and it was given to all the hospitals selected for the survey. Two days training was imparted for collection of cord blood sample to the paramedical staffs of the respective hospitals. The training was given by the research scholar along with other research team members.

Biochemical estimation: Umbilical cord blood collection

Cord blood was collected before placental delivery within five minutes after birth to avoid clotting. One drop of blood was applied to filter paper. The spots were dried at room temperature and the filter papers were sealed and kept in a freezer until assayed in the laboratory. The samples were stored at 4° C before analysis. The samples were estimated for TSH by using sandwich enzyme linked immuno-sorbent assay (ELISA) method (Slazyk and Hannon, 1993; Westgard and Klee, 1999).
METHOD AND MATERIALS

Inclusion and Exclusion Criteria

Inclusion criteria:

a) The Births occurring in the Hospitals/CHCs/PHC/ Institutions during the study period

Exclusion criteria:

a) Newborns with delivery in which iodine preparations have been used
b) Cesarean Deliveries
c) Pregnant Mothers who were on anti-thyroid therapies.

Sample Size: The sample size of 541 was estimated keeping in view of prevalence of neonatal hyperthyrotropinemia as 2.9%, confidential interval of 95% and relative precision of 2.0 and design effect of 2. However, we included 649 (Udham Singh Nagar), 670 (Nainital), and 694 (Pauri) of Neonates.

Repeat survey for Neonatal Hypothyroidism

Neonates with TSH levels 20mIU/l and higher were identified and were recalled for reassessment of TSH levels. The venous blood was utilized for repeat TSH estimation. The venous blood sample was taken on a filter paper. All the samples were estimated for TSH by using the sandwich enzyme-linked immunosorbent assay. The Neonates with TSH level more than 10mIU/l were identified to be suffering from Neonatal Hypothyroidism.
FLOW CHART

Uttarakhand State

3 regions

Terai  Kumaon  Garhwal

3 districts

Udham Singh Nagar  Nainital  Pauri Garhwal

using PPS Methodology

30 Clusters (Villages) from each district

Hospitals/CHCs/PHC/Institutions were identified and enlisted

6 Hospitals were selected from each district

450 births occurring in these Hospitals during the 12 months of study period were included

Umbilical cord blood collection on Filter paper

Estimation of Thyroid Stimulating Hormone (For Iodine Deficiency)

(By sandwich enzyme linked immuno-sorbent assay (ELISA) method

Repeat Survey for neonates with TSH level more than 20mIU/L for Neonatal Hypothyroidism

Neonates with TSH level more than 10mIU/L were diagnosed as Neonatal Hypothyroidism
Picture 1: Training of Paramedical Staff
METHOD AND MATERIALS

3.3 Part 3: Iodine Nutritional Status amongst School Age Children

Study Population: The study was carried on school age children (6-12 years) using school based approach

Study Area: Three Districts of Uttarakhand namely: Udham Singh Nagar, Nainital and Pauri Garhwal

Sampling technique: In the districts selected, 30 clusters (schools) were identified by utilizing population proportionate to size sampling (PPS) methodology recommended by WHO (2007) for IDD survey. In each cluster (schools), 60 children, in the age group of 6-12 years of age were covered. The children were covered by school based approach as more than 95% children attended the schools. All the primary schools in rural and urban area in the district with their respective child enrollment were enlisted. In each school, the children were briefed about the objectives of the study and the informed consent was undertaken. The date and time for the survey was decided as per the convenience of the school. In each identified school (cluster), 60 children were included using a Random Number Table (RNT). In each school, children were serially arranged according to their age groups 6-<8, 8-<10 and 10-12 years. With the help of the RNT, a total of 20 children were selected from each of the age group. If the desired sample of children could not be covered from the selected school, the nearest adjoining school was included to complete the sample size.

Clinical Examination

The clinical examination of thyroid gland was conducted by Palpation Method and graded as recommended by WHO (WHO, 2007). The grading of goitre was done according to the criteria recommended jointly by WHO/UNICEF/ICCIDD (a) Grade 0- not palpable and not visible; b) Grade I- palpable but not visible; and c) Grade II- palpable and visible). When in doubt, the immediate lower grade was recorded. The intra- and inter-observer variation was controlled by repeated training and random examinations of goitre grades. The sum of Grade I and II provided the Total Goitre rate (TGR) in the study population (WHO, 2007).
Biochemical Parameter

From each cluster (school), causal urine samples were collected from 19 children selected randomly, with the help of RNT from the list of children enrolled for clinical thyroid examination. Plastic bottles with screw caps were provided to each child for the urine samples. The samples were stored in the refrigerator until analysis. The analysis was done within 2 months. The UIC levels were analyzed at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi. The UIC levels were estimated using the wet digestion method (Dunn et al, 1993).

Iodine content in the salt

From each cluster (school), eighteen children were selected randomly and were provided with auto-seal polythene pouches with an identification slip. Children were requested to bring four teaspoons of salt (about 20g) from their family kitchen and the iodine content of the salt samples was analyzed at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi. The iodine content of salt was estimated by standard iodometric titration method (Karmarkar et al, 1986).

Sample Size: The sample size was estimated keeping in view of prevalence of goiter as 15%, confidence interval of 90% and relative precision of 15% and design effect of 2, a sample size of 1800 children was calculated to be covered from each district. However, we included 1807 (Udham Singh Nagar), 2269 (Nainital), and 2067 (Pauri Garhwal) of school age children.

Inclusion and Exclusion Criteria

Inclusion criteria:

a) Children of 6-12 years of age.
b) Subjects who have given the verbal consent

Exclusion criteria:

a) Children not attending the school on the day of survey
FLOW CHART

Uttarakhand State

3 regions

Terai

Kumaon

Garhwal

3 districts

Udham Singh Nagar

Nainital

Pauri Garhwal

using PPS Methodology

30 Clusters (Schools) from each district

60 School age children (6-12 years) from each school

Clinical Examination (n=60/school)

Biochemical Parameter (n=19/school)

Iodine content in the salt (n=18/school)

Goiter (By Palpation Method)

Urinary Iodine Concentration (By Wet Digestion Method)

Iodine Content in salt (By Titration Method)
3.4 Part 4: Iodine Nutritional status amongst Adolescent Girls

**Study Population:** The study was carried on Adolescent girls (12-18 years) using school based approach

**Study Area:** Three districts of Uttarakhand namely: Udham Singh Nagar, Nainital and Pauri Garhwal.

**Sampling Technique:** In the districts selected, 30 clusters (Schools) were identified by utilizing population proportionate to size sampling (PPS) methodology recommended by WHO (2007) for IDD survey.

In districts Udham Singh Nagar, Nainital and Pauri Garhwal the school enrollment of 12-18 years adolescent girls was more than 90 percent hence adolescent girls studying in school were considered the representative of the population in the area. Thus school based approach was adopted. All the senior secondary schools in rural and urban area in the district with their respective child enrollment were enlisted. Thirty schools (clusters) were selected according to population proportionate to size cluster sampling methodology. In each school, the girls were briefed about the objectives of the study and the informed consent was undertaken. The date and time for the survey was decided as per the convenience of the school. In each identified cluster (schools), 60 adolescent girls were selected. In each school, all girls in 12-18 years of age was serially arranged according to their age groups 12-<14, 14-<16 and 16-18 years. With the help of Random Number Table (RNT), 20 girls were selected from each of the age group. If the desired sample of adolescent girls could not be covered in the selected school, the nearest adjoining school was included to complete the sample size.

**Clinical Examination**

The clinical examination of thyroid of each adolescent girl was conducted. The grading of the goiter was done according to the criteria recommended jointly by WHO/UNICEF/ICCIDD (a) Grade 0- not palpable and not visible b) Grade I- palpable but not visible c) Grade II- palpable and visible). When in doubt, the immediate lower grade was recorded. The intra and inter observer variation was
controlled by repeated training and random examinations of goiter grades. The sum of Grade I and II provided the TGR of the study population (WHO, 2007).

**Biochemical Parameter**

From each cluster, casual urine samples were collected from 20 adolescent girls selected randomly, with the help of RNT from the list of girls enrolled for clinical thyroid examination. Plastic bottles with screw caps were provided to each adolescent girl for the urine samples. The samples were stored in the refrigerator until analysis. The analysis was done within 2 months. The UIC levels were analyzed at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi. The UIC levels were estimated using the wet digestion method (Dunn et al, 1993).

**Iodine content in Salt**

For collection of salt samples, 20 adolescent girls were selected randomly from each school and were provided with auto seal polythene pouches with an identification slip. Adolescent girls were requested to bring four teaspoons of salt (about 20g) from their family kitchen and the iodine content of the salt samples was analyzed at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi. The iodine content of salt was estimated by standard iodometric titration method (Karmarkar et al, 1986). Since iodine crystals are smaller than salt crystals and hence they get settled at the bottom of the pack. Thus, adolescent girls were instructed to mix the sample of salt properly before sampling.

**Sample size:** Keeping in view the anticipated prevalence of 11.6%, a confidence level of 95%, absolute precision of 1.5%, a sample size of 1751 was calculated to be covered from each district. However, 1823 (Udham Singh Nagar), 1811 (Nainital) and 1796 (Pauri Garhwal) of adolescent girls from each district were included.

**Inclusion and Exclusion Criteria**

**Inclusion criteria:**

a) Subjects who has given the verbal consent  
b) Girls of 12-18 years of age and Girls who were willing to give the samples

**Exclusion criteria:**

a) Adolescent girls not attending school on the day of survey  
b) Adolescent girls who are menstruating on the day of the survey
FLOW CHART

Uttarakhand State

3 regions

Terai

Kumaon

Garhwal

3 districts

Udham Singh Nagar

Nainital

Pauri Garhwal

using PPS Methodology

30 Clusters (Schools) from each district

60 Adolescent girls (12-18 years) from each school

Clinical Examination
(n=60/school)

Biochemical Parameter
(n=20/school)

Iodine content in the salt
(n=20/school)

Goiter
(By Palpation Method)

Urinary Iodine Concentration
(By Wet Digestion Method)

Iodine Content in salt
(By Titration Method)
3.5 Part 5: Environmental Factors

In the districts selected, 30 clusters (Villages) were identified by utilizing population proportionate to size sampling (PPS) methodology recommended by WHO for IDD survey. In the identified 30 clusters (villages), all the Hospitals/CHCs/PHC/ Institutions selected for Iodine deficiency in neonate were further selected for collection of water and food samples. Six clusters (Villages) were selected for collection of water and food samples from each district. Five samples from each village were collected. A total of 30 water and 15 food samples from each district was collected and analyzed for its iodine content.

**Estimation of iodine content in Water**

A total of 30 water samples from each district were collected from shallow tube wells (150-250 feet depth), dug wells, ponds, and river for estimation of iodine content in the water samples. These water samples was collected in screw capped plastic bottles and were stored at 4°C until analysis. The iodine content of water samples was analyzed by the research scholar along with the Laboratory Technician at the Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi by the wet Digestion method (Dunn et al, 1993).

**Estimation of iodine content in Food**

A total of 15 food samples from each district were collected in an auto seal pouches. Locally grown food by the farmers and stored by them like rice, wheat and pulses was collected and analyzed for its iodine content. These food samples were sent to National Institute of Nutrition, Hyderabad for the analysis of its iodine content. The iodine content in food samples was measured by an inductively coupled plasma mass spectrometry (ICP-MS) method (Fecher et al, 1998). To avoid the effect of seasonal variation the samples were collected within a short span of time.
15 Food (cereals and pulses) and 30 Water Samples were randomly collected from each district.

**Flow Chart**

**Uttarakhand State**

- 3 regions
  - Terai
  - Kumaon
  - Garhwal

- 3 districts
  - Udham Singh Nagar
  - Nainital
  - Pauri Garhwal

- Food Samples (By Karmakar et al method, 1986)
- Water Samples (By Karmakar et al method, 1986)

- National Institute of Nutrition, Hyderabad
- AIIMS, New Delhi
METHOD AND MATERIALS

Picture 2: Collection of Water samples from Shallow Tube Wells

Picture 3: Collection of locally grown food samples
Table 12: Summary of sample size of major indicators

<table>
<thead>
<tr>
<th>District</th>
<th>Study Group</th>
<th>Clinical Examination</th>
<th>Biochemical</th>
<th>Iodized salt intake</th>
<th>TSH</th>
<th>Water sample</th>
<th>Food Sample</th>
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<tbody>
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<td>Udham Singh Nagar</td>
<td>Pregnant Mothers</td>
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<td>532</td>
<td>597</td>
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<td>30</td>
<td>15</td>
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<td>Neonates</td>
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<td></td>
<td>Adolescent girls</td>
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<td>748</td>
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<td>548</td>
<td>-</td>
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<td>15</td>
</tr>
<tr>
<td></td>
<td>Neonates</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>670</td>
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<td>642</td>
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<td></td>
<td>Adolescent girls</td>
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<td>600</td>
<td>608</td>
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<tr>
<td></td>
<td>Adolescent girls</td>
<td>1796</td>
<td>594</td>
<td>600</td>
<td></td>
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</tr>
</tbody>
</table>

3.6 Data Management

Data was collected and entered in excel sheet. The entire data in the excel sheet was checked for its validity. After that the data was cleaned, it was freeze and subjected to statistical analysis. The tables were developed after the statistical analysis was undertaken.

3.7 Statistical Analysis

Data was analyzed by strata 12 and presented in Mean (S.D), median, minimum, maximum, frequency and percentages. Continuous variable were compared among the group by Wilcoxon rank-sum (Mann-Whitney) test (2 groups)/ Kruskal-Wallis (more than 2 groups), followed by multiple comparison using Wilcoxon rank-sum (Mann-Whitney) test with Bonferroni.

Categorical variable were compared by chi square or Fisher's exact test. Correlation between the two continuous variable was assessed by spearman's correlation coefficient. P value of <0.05 was taken as statistically significant.
3.8 Details of the methods utilized

- Clinical examination of Goiter (Appendix-1)
- Biochemical estimation: Urinary Iodine Concentration level, Wet Digestion (Appendix-2)
- Thyroid Stimulating Hormone: Dry blood spot in filter paper, Sandwich enzyme linked immunosorbent assay (Appendix-3)
- Iodine content in the salt: Iodometric Titration method (Appendix-4)
- Iodine content in Water: Method of Karmakar et al, 1986 Calorimetrically (Appendix-5)
- Iodine Content in Food samples: Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method (Appendix-6)
- Permission letters: (Appendix-7)
- Porforma: (Appendix-8)
- Personal Information Sheet (PIS): (Appendix-9)
- Participant Informed Consent Form (PICF): (Appendix-10)