CHAPTER 5
SUMMARY AND CONCLUSION

PHASE I
EFFICACY OF DOUBLE FORTIFIED SALT SUPPLEMENTATION AMONGST PREGNANT WOMEN

SUMMARY

Introduction

Globally iron and iodine deficiencies affects one third of the total population. However, the pregnant women and their fetuses are most affected by these deficiencies. Iodine is an essential micronutrient for the synthesis of thyroid hormones, which regulates the metabolic pattern of most cells and plays a vital role in the process of early growth and development of most organs, especially the brain. Iodine deficiency at critical stages of foetal life and early childhood remains single most important and preventable cause of mental retardation globally (WHO/ICCIDD/UNICEF 2007). However, iron plays a vital physiological role in growth, development, metabolic reactions, cofactor for most of the enzymatic activities and cognitive development of the fetus. Untreated chronic/severe maternal IDA may prove to be detrimental to the developing fetus. The prevalence of anemia amongst Indian pregnant women is more than 60% (NFHS-III. 2005-2006).

Methodology

The present research phase was undertaken with the broad objective “To study the impact of double fortified salt supplementation amongst pregnant women”

Sample selection and site of the phase I

A semi-government hospital - having antenatal clinic with higher number of inflow of pregnancy registrations and an accountable delivery rate (250-300/month) throughout the year in the centre of the Vadodara city was selected as a study site. Pregnant women, who were attending antenatal services at the study site were selected and enrolled from the study setting.
This phase was designed as a hospital based longitudinal study including experimental-control samples, where impact of two types of intervention (given throughout gestation) was compared.

1. Double fortified salt supplementation to the experimental group and counseling on consuming iodized salt was provided to the control group
2. Nutrition health education was provided to both the groups.

Prior permission for the study was obtained from the hospital authorities. Written consent from the pregnant women, at the time of registration was availed.

**Experimental design of the phase I**

This phase was divided into 3 major sections:

**Section I: Baseline survey**

Data from (N=256) all enrolled pregnant women was collected on their socio-economic status, anthropometry parameters, hemoglobin concentration, urinary iodine concentration and thyroid analytes.

**Specific objectives:**

- Screening of pregnant women from urban Vadodara for iodine and iron deficiencies
- To assess their nutritional status through anthropometry standards
- To assess their socio-economic status

Total enrolled population (N=256) at the first visit were subsampled N=150 owing to drop out rate due to various reasons such as migration to maternal place or other place, abortions, miscarriages, unwillingness to participate, family disagreement and non-reachability.

Further, baseline data was screened to maintain the homogeneity of the enrolled population and have a representative scenario of the maternal health belonged to LIG in Vadodara city.
The sample size and methods are summarized in Table 5.1. Before and after intervention, data of the study groups were collected on nutritional status, hemoglobin concentration, urinary iodine concentration, thyroid analytes, cord blood samples, KAP, dietary intake etc. were assessed and results were recorded.

Pregnant women during first trimester (<15 weeks) of gestation, singleton pregnancy and without visible signs of hypothyroidism were included in the study.

Section II: Interventional strategies

This section comprised of the interventional strategies to be implemented upon our study subjects- pregnant women. These strategies included,

1. Double fortified salt supplementation amongst experimental group and advocacy for optimally iodized salt consumption amongst control group.
2. Nutrition Health Education to the pregnant women belonged to both the groups.

Table 5.1: Study indicators and tools for data collection

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Indicators</th>
<th>Tool</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Socio-economic status</td>
<td>Structured questionnaire</td>
<td>256</td>
</tr>
<tr>
<td>2</td>
<td>Anthropometry indices</td>
<td>Standard methods</td>
<td>256 (Baseline) 121 (Final)</td>
</tr>
<tr>
<td>3</td>
<td>Hemoglobin Estimation</td>
<td>Cynmet-hemoglobin method</td>
<td>256 (Baseline) 121 (Final)</td>
</tr>
<tr>
<td>4</td>
<td>Urinary iodine excretion</td>
<td>Sandell-kolthoff reaction(Modified microplate technique)</td>
<td>256 (Baseline) 121 (Final)</td>
</tr>
<tr>
<td>5</td>
<td>DFS content estimation</td>
<td>BIS standards</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Thyroid hormones-TSH, FT₄,TT₄,Tg</td>
<td>RIA technique</td>
<td>256(Baseline) 121 (Final)</td>
</tr>
<tr>
<td>7</td>
<td>Cord blood Analysis-TSH, FT₄,TT₄,Tg</td>
<td>RIA technique</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>Dietary intake- 24 hr. dietary recall and FFQ</td>
<td>Structured questionnaire</td>
<td>121 (Baseline and Final)</td>
</tr>
<tr>
<td>9</td>
<td>Knowledge, attitude and practices</td>
<td>Semi-structured questionnaire</td>
<td>121 (Baseline and Final)</td>
</tr>
</tbody>
</table>

The specific objectives of this section were,
• To Supplement DFS to the experimental group and counsel control group to consume optimally iodized salt
• Monitoring salt iodine content of the household samples of pregnant women belonged to control group
• Provide nutrition health education regarding iodine and iron nutrition, best practices for storage and usage of DFS/iodized salt to the pregnant women belonged to both the groups
• To collect blood samples for Hb estimation, urine samples for urinary iodine concentration and serum samples to analyze thyroid hormones at the end of each trimester
• To monitor the compliance of DFS consumption and provide counseling whenever problem in the usage aroused.

Out of (N=150) sub grouped subjects, experimental and control group were formed by incorporating n=75 pregnant women to each group randomly. With the progression of gestation and our study phase, 121 pregnant women made the final number of the subjects. Of which N=67 from experimental and N=54 from control group completed the study.

**Section III: Impact assessment**

This section was formed for assessment of DFS supplementation and non supplementation amongst pregnant women of both the groups using various indicators for iodine, iron and nutritional status. Impact on pregnancy outcome and neonatal parameters were also included in this section to assess the extent of efficacy of DFS giving health benefits.

There was also an assessment on the impact of NHE on knowledge, attitude and practices of the subjects, their dietary habits (quantity and quality) and frequency as well.

The specific objectives of this section were:

➢ To study the impact of DFS supplementation and non supplementation on various indicators:
✓ on nutritional status
✓ on the prevalence of anemia
✓ on prevalence of iodine deficiency disorders and iodine status
✓ on pregnancy outcome
✓ on neonatal anthropometry parameters
✓ on neonatal thyroid analytes

➢ To compare the relative impact of NHE on
✓ 24 hr. dietary recall
✓ FTQ
✓ KAP

Post intervention data was collected with regards to change in hemoglobin concentration, urinary iodine excretion, thyroid analytes, nutritional status, dietary intake, KAP, neonatal anthropometry and neonatal thyroid analytes.

Data Analysis

Mean, median and standard deviations were calculated for maternal height, weight, age, BMI, hemoglobin concentration, urinary iodine concentration, thyroid analytes, dietary intake and neonatal height, weight, head circumference, gestational age, Z scores (WHO growth standards) and thyroid analytes. 95% CI and percentiles (95th and 5th) were revealed wherever required. Correlation coefficients used for assessing correlation between different indicators. Paired 't' test, chi square and independent 't' test were used to assess significant difference within and between groups. Minimum level of significance was kept at p<0.05. Post hoc analyses were carried out whenever further analytes on data reevaluation was required.

MAJOR FINDINGS OF PHASE I

5.1 BASELINE SURVEY

At baseline, a survey on assessment of general characteristics, nutritional status, iodine and iron status of the pregnant women enrolled was carried out.
> Forty four percent of the subjects were primiparous and rests of them were multiparous. All the subjects were enrolled during first trimester. Majority were enrolled towards the end of first trimester (68.53%)
> Thirty five percent of the subjects were underweight at the time of registration and half of the subjects were in the normal category of BMI.
> There were 90% subjects with iron deficiency anemia at the time of registration and majority were moderately (62.1%) anemic. Mean hemoglobin concentration was 9.26 g/dl depicting the population as moderately anemic.
> The median urinary iodine excretion (297.14 μg/L) showed that population was iodine sufficient at the time of enrollment in all the 3 months of first trimester. However, there were 16.79% of the subjects, who had insufficient UIE (<150 μg/L).
> Mean TSH, FT₄ and TT₄ were observed to be in normal range for the population. A reciprocal pattern (r= -0.178, p<0.01) between TSH and FT₄ levels was observed as normal pregnancy induced physiological fluctuations.
> Inter relation between anthropometry parameters and hemoglobin concentration revealed significant correlation (height= p<0.05 and weight-BMI= p<0.01). However, UIE did not give any correlation with any of the parameters.
> Month of gestation during first trimester correlated negatively (r= -0.248, p<0.01) with FT₄ levels.
> More than 70% of the subjects were consuming optimally iodized salt (iodine content ≥ 15 ppm).
> Sub grouped population (N=150) also revealed a more or less similar set of results with regards to above indicators and represented the enrolled population.

5.2 INTERVENTIONAL STRATEGIES AND MONITORING

5.2.1 Distribution of the population into groups

> The sub grouped population was divided equally into experimental (n=75) and control (n=75) groups randomly.
> The experimental group was subjected to DFS supplementation and control group was advocated to consume adequately iodized salt.
5.2.2 DFS as a supplement

- Double fortified salt was used as a supplement for the pregnant women, who were grouped into experimental subjects.
- Before supplementation, iodine and iron content of DFS were assessed using titrimetric method (BIS standards). Mean iodine content was 40 ppm (recommended 30 ppm) and iron content was 1050 ppm (recommended 1000 ppm) (Recommended by BIS 1999, GOI/WHO 2004).
- On initiation and during supplementation process, experimental group was provided ample advocacy regarding the usage and storage of DFS towards increasing acceptability and stability of the contents.
- DFS was very widely accepted by the subjects (95.5%) and their family members (82.1%). Initially 70.1% of the subjects observed colour change in the food preparations after cooking, but the percentages could be reduced by providing counseling on the issue.

5.2.3 Nutrition Health Education as a tool

- NHE was provided to both the groups regarding iodine and iron nutrition, storage and usage in cooking practices for DFS/iodized salt to avail optimum iodine from the salt.
- Control group was counseled for iodized salt and iodine rich foods consumption to have a comparable iodine intake to the experimental group.

5.3 IMPACT ASSESSMENT ON THE PARAMETERS

5.3.1 Impact on nutritional status

- There was an improvement in nutritional status of the subjects compared to the stage of registration. This improvement was reported in terms of BMI (kg/m²). There was a significant (p<0.001) gradual reduction in the percentage of underweight subjects with the progression of gestation. However, there was no significant difference observed in BMI distribution of both the groups.
- With the observed change in percent distribution in BMI categories, there was also a significant difference observed in mean BMI within groups but no difference was
observed between both the groups. There was a similar effect of NHE observed in both the groups.

5.3.2 Impact on iron status

- All the subjects were classified according to WHO recommendation on classification for anemia, 2001 and Shobeiri et al 2006 with trimester specific cutoffs during pregnancy. Based on their percent distribution during first trimester results, 90% of the subjects were anemic in experimental and 85.2% in control group.

- At the end of gestation, 1.5% increase in proportion of the normal subjects (nonanemic- from mild to normal) of experimental group and 11.1% reduction in normal subjects of control group was reported. However, both the groups were on IFA supplements, DFS showed stability in the hemoglobin levels of the non anemic subjects but still there was a remarkable difference in both the groups for normal category.

- At the end 50% and 25% of the subjects in remained mildly anemic in experimental and control group respectively. However, the positive shift in the experimental group and negative shift in the control group was observed in the classification of anemic subjects.

- During second trimester there was non significant reduction (p>0.05) in Hb concentration of experimental group and significant reduction (p<0.05) in Hb concentration of control group.

- During third trimester, the Hb concentration improved significantly in experimental group compared to both the trimesters (p<0.01). However, non significant improvement in control group was observed.

- There was a significant improvement in mean hemoglobin concentration amongst experimental group compared to control group (p<0.05).

- This in turn suggests the beneficial effect of DFS as a dietary source of iron compared to experimental group.
5.3.3 Impact on iodine status

All the subjects were classified according to the WHO, ICCIDD,UNICEF recommended classification for iodine deficiency disorders, 2007 during pregnancy. Based on their percent distribution during first trimester results 14% of the subjects were iodine deficient and the percentage remained same till the end. There were 16.4% of the subjects in experimental group and 11.1% subjects in control group as iodine insufficient based on the urinary iodine levels during first trimester.

- Median urinary iodine levels were non significantly different on comparing both the groups. Significant increase in median urinary iodine of experimental group during second trimester was observed (p<0.05). On comparing with first trimester urinary iodine levels decreased significantly towards the end (p<0.05).
- However, urinary iodine levels decreased gradually in control group. The difference was non significant between first and second trimester and it was significant between first and third trimester (p<0.05).
- It can be stated that there was no significant difference between both the groups using urinary iodine as an indicator, which could be achieved, since majority of the subjects were on optimal iodine intake. This in turn reveals a successful functioning of salt iodization programme and impact of NHE to meet the optimal iodine nutrition in non supplemented group compared to DFS group.

5.3.4 Impact on thyroid analytes

- Mean serum TSH and FT4 did not vary significantly throughout gestation. However, within group variation for FT4 was observed among the subjects belonged to control group (p<0.05).
- Mean TT4 level improved significantly in both the groups during third trimester (p<0.001), which is a physiologically driven condition and DFS did not play a significant role to bring an influence on hormone levels.
- Based on the urinary iodine deficiency and sufficiency classification, thyroid analytes were assessed for both the groups. It was observed that, the subjects with iodine deficiency based on first trimester UIE levels showed higher levels of TSH and lower
levels of FT₄ compared to iodine sufficient subjects. This difference was observed throughout gestation.

- Baseline assessment of thyroid hormones recommended by “The Endocrine Society” 2007 cutoffs for TSH: recommendations provided by commercial kits for FT₄ and TT₄, the subjects were classified with SCH (17.91%-experimental and 27.77%-control group), OH (11.29%-experimental and 9.26%-control group), Hypothyroxinemia (40%-experimental and control) and Euthyroidism (36.36%-experimental and control).

5.3.5 Impact assessment with pregnancy outcome

- There was no significant difference on neonatal parameters between both the groups was observed. It was observed that, none of the maternal parameters correlated with neonatal parameters.

- There were 20.48% of the neonates, who were born with low birth weight (<2.5 kg) irrespective of groups for both the groups and 13.25% of the neonates born premature (<37 weeks of gestation).

- Maternal hemoglobin at 3rd trimester correlated significantly with neonatal birth weight (p<0.05). This in turn suggests the importance of normal hemoglobin concentration during pregnancy.

- Neonatal TSH showed a significant correlation with LBW. Odd’s ratio and 95% CI limits were (3.55 : 0.97,13.44). Relative risk of LBW was higher in neonates with cord blood TSH >10 µIU/ml or vice versa.

- Using WHO growth standards, nutritional status of the neonates was assessed. It revealed that, 27.71% of the subjects were moderately and 19.28% were severely stunted. Moderate underweight was observed in 10.85% and 7.22% were falling in severely wasted class.

5.3.6 Impact assessment with dietary intake

- The mean energy, protein, CHO and iron consumptions were comparatively lower in both the groups with deficit percent as 49.42%, 62.31%, 10.45% and 84.42%
respectively for experimental group. However, these deficit percentages were 52%, 64.34%, 14.07% and 85.97% respectively for control group.

- After imparting NHE as an interventional strategy, data on mean intake of energy, protein, CHO and iron was recollected during third trimester and a significant difference was observed for each nutrient in both the groups.

- The differences for energy, protein and CHO were approximately 10-11%, 10-12%, 20% respectively for both the groups. However, iron intake improved significantly in both the groups \( (p<0.001\text{-experimental and } p<0.01\text{-control}) \). Iron intake improved significantly \( (p<0.01) \) in experimental group compared to control group due to DFS consumption also.

- Overall food frequency did not show any significant change in both the groups. However, change in the consumption frequency of the recommended iron and Vitamin C rich foods was observed amongst both the groups as a success of NHE.

5.3.7 Impact on KAP

- There was a significant improvement on KAP of the subjects, which was observed in both the groups. An improvement in knowledge about iodine, iodized salt, storage and usage practices for DFS/iodized salt was observed after NHE.

- There was almost 40% increase in awareness regarding sources of iodine amongst experimental and control groups. Increase in knowledge regarding individual sources in both the groups ranged from 10-40%.

- In both the groups there were 70% of the subjects, who started recognizing iodized salt from its “Smiling sun logo” and 30% of the subjects with its label. Thus, 100% of the subjects became aware of the identification of the iodized salt from the packet.

- Almost 55-66% of the subjects started following best practice to maintain iodine in the preparation and majority of the subjects were using best practices of the storage of DFS/iodized salt.

- Majority of the subjects attained knowledge on consequences of IDD on physical and mental development of the growing fetuses and children.

- More than 85% of subjects could respond on various sources of iron and almost 80% were aware about specific consequences of iron deficiency anemia on maternal and fetal health.
Compliance to IFA and frequency of consumption could be improved by 30% amongst control group.

Increased impact on food intake (quantity) also was observed with 9% in experimental group and 35% in control group.

There was a significant reduction in food taboos and majority of the food items which were restricted due to blind believes, hence the consumption of these foods was initiated.

Thus, it could be stated that, providing NHE or parallel counseling, helps in improving knowledge levels of the populations to attain betterment in nutrition and health status.

**CONCLUSION AND UNIQUE CONTRIBUTION AS RECOMMENDATIONS OF RESEARCH PHASE I**

To conclude the present phase, validation and usefulness of double fortified salt for pregnant women and emphasizes the need for including DFS into life cycle approach. It is more effective when used with other supportive strategies such as improvised nutrition intakes based on nutrition health education/counselling.

The results of the study, viewed collectively, lead to the rejection of the hypothesis stated at the onset of the study (Described in methodology), which were as follows:

1. There is no significant impact of DFS supplementation (compared to control) on iron and iodine status.
2. There is no significant impact of NHE on nutrient intake and KAP of the subjects

Contrary to the hypothesis, the experimental group was highly benefited from DFS supplementation:

- Experimental group had an improvement in iron status and iodine status.
- The study population was iodine sufficient but neonatal parameters showed moderate iodine deficiency, so efforts should be targeted in maintaining iodine sufficiency.
➢ The hemoglobin status of the population calls for an immediate action to be improved since most of the subjects are anemic and hence, the prevalence of LBW is very high (20%).

➢ Among pregnant women from lower strata of the community, whether supplemented with IFA tablets or not, should be supplemented with DFS throughout gestation and even before and after gestation to have a sustained liberation of iron from the salt in the gut.

➢ Prevalence of thyroid dysfunction as SCH, OH and hypothyroxinemia is comparatively higher than the reviewed literature. There is a need for careful monitoring of the subjects and if necessary they should be put on hormone therapy, till they achieve normal levels.

➢ All pregnant women need to be screened for iodine deficiency and thyroid dysfunction as soon as the pregnancy is confirmed.

➢ Pregnant women and their concerned family members (whenever required) should be made aware about the importance of iodine and iron nutrition and their consequences resulted by their deficiencies during pregnancy or prepregnancy stage.

➢ Multiple approaches should be used to combat micronutrient deficiencies as an initiative and support strategies from all stake holders and the government for a permanent solution.
PHASE II
IMPACT ASSESSMENT OF DOUBLE FORTIFIED SALT
SUPPLEMENTATION AMONGST SCHOOL CHILDREN

SUMMARY

Introduction

Evidence based studies have reported that, physical growth and cognitive development in children are faster during early years of life, and that by the age of four years, 50% of the adult intellectual capacity has been attained and before thirteen years, 92% of adult intellectual capacity is attained (Vernon 1976). However, it is affected significantly due to micronutrient deficiencies, especially iron and iodine.

Thus, to combat these deficiencies NIN has ventured with a stable formula as Double fortified salt (DFS) to meet the major part of the RDA of iron and iodine requirements of the children during their growth spurt and pubertal stages. This phase is an effort to achieve the same amongst children belonged to endemic region for iron and iodine deficiencies.

Methodology

The present research phase was undertaken with the broad objective “To study the impact of double fortified salt supplementation amongst school children”

Sample selection and site of phase II

Waghodia, a rural block of Vadodara district was considered as a study site. According to State Nutrition Cell 2008-2009, Waghodia has 12.6% goiter prevalence, depicting it as an endemic region. Out of 172 primary government schools in the block, 4 schools were randomly selected on the same belt. Based on the percent availability of iodized salt, the schools were distributed into experimental (n=2) and control groups (n=2). All the school children from the standard 1st to 6th (5-15 years) made the final sample size N=1184, where N=947 could complete the study.
This phase was designed as an interventional longitudinal experimental-control study, where impact of DFS supplementation was compared and assessed for the initial and post interventional difference.

1. DFS supplementation to the experimental group was carried out. Counseling was provided to consume optimally iodized salt to the control group.
2. NHE and BCC to the children and their mother/caretakers was provided on iodine and iron nutrition, consequences of the deficiencies, storage and cooking practices using iodized salt and DFS.

Informed consent for the study was obtained from education officials, school principals and parents of the children. However, children were also explained the purpose of the study.

**Experimental design of the Phase II**

This phase was divided into 3 major sections.

**Section I: Baseline survey**

Data from (N=1184) children belonged to four purposively selected schools was collected on their anthropometry indices, hemoglobin concentration, urinary iodine excretion, thyroid analytes and IQ/cognitive scores.

**Specific Objectives:**

- To map the availability of iodized salt in the study area
- To map the prevalence of iodine deficiency and iron deficiency amongst school children from the selected schools.
- To assess baseline nutritional status through anthropometry indices.
- To assess baseline IQ and cognition scores of the study population.

Total N=1184 children were enrolled from the four schools of Waghodia block at baseline. Later the final sample size reached to N=947, since the rate of absenteeism was very high (30%) amongst rural school children. The exclusion criteria included the unavailability of the same child for 3 consecutive visits during supplementation period,
non reachability of the child or unwillingness of the children/parents to be a part of the study. The sample size and methods are summarized in Table 5.2.

Table 5.2: Study indicators and tools for data collection

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Indicators</th>
<th>Tools</th>
<th>Samples Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Socio-Economic Status</td>
<td>Structured Questionnaire</td>
<td>212 (Subsample)</td>
</tr>
<tr>
<td>2.</td>
<td>Anthropometry</td>
<td>Standard methods and tools</td>
<td>1184 (Baseline)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>947 (Final)</td>
</tr>
<tr>
<td>3.</td>
<td>Hemoglobin Estimation</td>
<td>Cynmet-hemoglobin method</td>
<td>972 (Baseline)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>947 (Final)</td>
</tr>
<tr>
<td>4.</td>
<td>Urinary iodine excretion</td>
<td>Sandell-kolthoff reaction (Modified microplate technique)</td>
<td>1034 (Baseline)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>947 (Final)</td>
</tr>
<tr>
<td>5.</td>
<td>DFS content estimation</td>
<td>BIS standards</td>
<td>3</td>
</tr>
<tr>
<td>6.</td>
<td>Thyroid hormones-TSH, FT₄, TT₄, Tg</td>
<td>RIA technique</td>
<td>212 (Baseline)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>189 (Final)</td>
</tr>
<tr>
<td>7.</td>
<td>Dietary intake- 24 hr. dietary recall and FFQ</td>
<td>Structured questionnaire</td>
<td>212 (Baseline)</td>
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<td></td>
<td></td>
<td></td>
<td>212 (Final)</td>
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<tr>
<td>8.</td>
<td>Knowledge, attitude and practices</td>
<td>Semi-structured questionnaire</td>
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<td>212 (Final)</td>
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<tr>
<td>9.</td>
<td>Household salt samples</td>
<td>Spot testing kit</td>
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<tr>
<td>10.</td>
<td>IQ and Cognition Tests-Draw-a-man, Visual Memory test, Clerical Test</td>
<td>Standardized methods</td>
<td>823-864 (Baseline)</td>
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<td></td>
<td></td>
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<td>700 (Final)</td>
</tr>
</tbody>
</table>

Before and after intervention, data of the study groups were collected on nutritional status, hemoglobin concentration, urinary iodine concentration, thyroid analytes, IQ and cognition scores, dietary intake and parental SES-KAP etc were assessed and the results were recorded.

Section II: Interventional strategies

This section comprised of the interventional strategies to be implemented on the study population-school children. These strategies included,

(1) Double fortified salt supplementation amongst experimental group and advocacy for optimally iodized salt consumption amongst control group.
(2) Deworming among half of the children belonged to both groups, which lead to final number of study groups to be four:

- Experimental and dewormed group (E+DW)
- Experimental group (E)
- Control and dewormed group (C+DW)
- Control group (C)

(3) Nutrition health education was provided to the mothers of the children belonged to all the groups, regarding iodine and iron nutrition.

**Specific objectives:**

- To classify study population into experimental and control based on the availability of iodized salt in the village (household and retail shop samples).
- To administer deworming tablets amongst both the groups, subdivision into 4 groups.
- To collect information on SES and KAP on the subsample.

Out of N=4 schools selected for the study phase, 2 schools to experimental and control group each, who were divided based on the availability of iodized salt. In control group, there were almost all population were consuming iodized salt (>15 ppm). However, the experimental group showed below 70% consumption of iodized salt. This observed difference in both the groups could have been the result of the distance from the city area. Since schools included in experimental group were 5-10 kms more into the deep of rural area than the control group school from the border of urban Vadodara.

**Section III: Impact assessment**

This section was comprised of impact assessment of DFS supplementation and non-supplementation amongst school children, along with and without deworming. The indicators included iodine and iron status, nutritional status and IQ/cognition tests.

There was also an assessment on the impact of NHE and BCC on the knowledge, attitude and practices of the mothers/caretakers of the children, dietary intake (quantity and quality) of the children and frequency as well.
The specific objectives of this section were:

- To study the impact of DFS supplementation and non supplementation on various indicators.
  - On nutritional status
  - On the prevalence of anemia
  - On the prevalence of iodine deficiency
  - On IQ/cognitive parameters
- To compare the relative impact of NHE on,
  - 24 hours dietary recall
  - FFQ
  - KAP (parental)

Post intervention data with regards to change in hemoglobin concentration, urinary iodine excretion, thyroid analytes, nutritional status, dietary intake, IQ/cognitive parameters, KAP of the mothers etc. was collected.

**Data analysis**

Simple descriptive analysis of the data was carried out. Statistical analysis was performed using Chi-square ($X^2$) when appropriate for categorical data. Normal distribution of the data was assessed by the Kolmogorov-Smirnov test. Where indicated, the data was normalized using log transformation to facilitate the use of normal-theory analytic methods. Nonparametric (Mann-Whitney U test, Kruskal-Wallis test and Wilcoxon rank test) or parametric (Student’s t-test, paired t test and ANOVA) statistical tests, depending on the normality of the data, were used to detect within-group and between group differences. Further post-hoc Bonferroni analysis was carried out. To determine associations between analytes Pearson’s correlation or Spearman’s rank correlation were calculated. A two-tailed p values <0.05 was considered statistically significant.
MAJOR FINDINGS OF PHASE II

5.4 BASELINE SURVEY

At baseline, a survey on assessment of anthropometry indices, iodine and iron status, IQ and cognitive scores of the school children was carried out.

- Baseline data on anthropometry revealed mean height and weight of the children were 122.80±12.75 cm and 21.21±6.20 kg. Data on malnutrition based on CDC standards revealed 44.60% stunting, 70.78% underweight and 54.16% thinness in the study population. There was a significant difference for WAZ score observed between both the genders.

- Mean hemoglobin concentration of the population was observed to be 9.17±1.23 g/dl, indicating population into moderately anemic category. However, gender wise significant difference was observed with hemoglobin concentration at 9.27 g/dl and 9.04 g/dl amongst boys and girls respectively.

- According to WHO classification, overall prevalence of anemia was 98%, with majority of the children to be moderately anemic (70%) and 24% with mild anemia.

- Median UIE of the population was 146.33 μg/L, indicating the population to be iodine sufficient. There was no significant difference observed between both the genders.

- However, there were 30% of the children who were iodine insufficient, including 18.2% mild and 8.5% moderately deficient. There were 3% of the children with severe deficiency also. The classification followed was recommended by WHO/ICCIDD/UNICEF, 2007.

- Based on thyroid analytes assessment, normal range for TSH, FT$_4$ and TT$_4$ were observed amongst the study population. Incidence of subclinical hypothyroidism was observed amongst 2.4% children.

- Analysis of IQ and cognition test results revealed that, the population was below average IQ scores (Draw- a man test) using standard classification recommended by Phatak P (2002) and Wechsler’s scale. However, mean score for Visual memory test (VMT) was 0.40 and 0.48 amongst boys and girls respectively, where as it was 0.72 and 0.76 for Clerical test (CT) for both the gender respectively.
VMT scores varied significantly based on the presence of iron deficiency anemia where it was observed that, the anemic population (moderate-severe) scored lower than the normal-mild anemic). When the results were compared based on the gender-wise category, higher scores were observed among girls compared to boys. Similar pattern was also observed for the presence of iodine deficiency.

Nutritional status classification (deficient and normal) of the children cross tabulated mean hemoglobin concentration showed a significant difference for HAZ and WAZ. However, the difference was non significant for iodine deficiency. This indicates hemoglobin concentration varies with the nutritional status, where as UIIE does not depends upon the Z scores.

5.5 INTERVENTIONAL STRATEGIES

5.5.1 Distribution of the population into study groups

The study population was divided into two groups, as experimental and control for double fortified supplementation as a tool. These groups were further subdivided as dewormed and non dewormed groups, resulting in two subgroups into each study group. This was done to assess the impact of DFS with deworming compared to deworming alone. Hence, final number of groups was four: E+DW, E, C+DW and C groups.

5.5.2 Supplementation

Before supplementation, iodine and iron content of DFS were assessed using titrimetric method (BIS standards). Mean iodine content was 40 ppm and iron content was 1050 ppm.

On initiation of DFS supplementation amongst experimental groups (E+DW and E), control groups (C+DW and C) were recommended to consume optimally iodized salt. The supplementation continued for 9 months.

SES of the study groups was collected, data revealed that, majority of the children were Hindus, with majority belonged to joint families. Parental literacy rate revealed that, majority of the mothers of the children were illiterate or educated till primary
school. However, fathers of the children were educated till primary or secondary school.

- Majority of the mothers were housewives, one fifth of the percent fathers of the children were doing Jobs and half of the total were on labor contracts, classifying majority of the families into lower income groups.
- Based on the data on KAP and dietary intake, NHE to all the groups was provided on iodine and iron nutrition, their importance in their children’s health, cooking and dietary modification for optimal gain of the nutrients, consequences of these micronutrient deficiencies, consumption of iodine and iron rich foods etc were provided throughout the study period.

5.6 IMPACT ASSESSMENT ON THE INDICATORS

5.6.1 Impact on nutritional status
- Impact on nutritional status was observed among all the study groups. Since there was almost 3.4 kg weight gain within 1 year in the children. However, height increased to 6.3 cm including all the study groups. These increases were significant in all the study groups (p<0.001).

- However, there was significant difference between groups for difference in height, which varied significantly between E+DW and rest of the groups, indicating significant impact of DFS on height gain of the children compared to their counterparts in rest of the groups. This resulted due to highest increased height in E+DW compared to rest of the groups. However, the increase in weight varied significantly between C+DW and rest of the groups, since C+DW showed lowest weight gain. The increased weight and height could have been due to the growth spurt of the children and slightly favored by iron source from DFS. Overall prevalence of malnutrition (lower HAZ, WAZ and BMZ) decreased significantly with growth spurt of the children.

5.6.2 Impact on micronutrient status

5.6.2.1 Impact on iron status
- Mean hemoglobin concentration of experimental group (+0.42 g/dl) increased significantly (p<0.001) compared to decrease (-0.54 g/dl) in control group. At baseline
there was a significant difference between both the groups (p<0.001), however the difference has remained non significant towards the end.

➢ When mean hemoglobin improvement was compared for all 4 study groups, a significant (p<0.001) improvement among E+DW group was observed with 0.60 ±1.09 g/dl was observed and also among E group with 0.21 ± 1.04 g/dl at level of significance with (p<0.01).

➢ On the contrary, there was a significant reduction in hemoglobin concentration of both control groups (C+DW and C) with mean -0.5 g/dl.

➢ Prevalence of mildly anemic subjects increased in experimental groups with the positive shift of moderately anemic children in the category. However, control groups showed negative shift with increased percentage of moderately anemic children.

➢ Total prevalence of anemia decreased by 6.3% in E+DW group, where as it increased by 1.5%, 22.5% and 21.0% in E, C+DW and C groups respectively.

5.6.2.2 Impact on iodine status

➢ Median UIE of the both the groups was above 100 µg/L, which is a cutoff for classifying the population into iodine sufficient category at baseline. This was reflected by UIE at 132.13 µg/L and 158.18 µg/L for experimental and control group respectively.

➢ Experimental group were supplemented with DFS, which replaced the iodized salt in the area, where as consumption of optimally iodized salt was emphasized for the children belonged to control group. Further, post intervention data on UIE revealed median levels to be 177.02 µg/L and 238.22 µg/L for experimental and control groups respectively. The improvement was observed to be significant at higher level (p<0.001) for both the groups.

➢ Proportion of iodine sufficiency increased for all groups with significant reduction (p<0.001) in iodine insufficiency for all four groups. Percent reduction of iodine insufficiency was observed to be 24.3%, 20.3%, 20.6% and 13.8% for E+DW, E, C+DW and C groups respectively.

➢ This ended with improved percentages of normal children in E+DW, E, C+DW and C group with 84.9%, 88.2%, 93.5% and 91.8% respectively. This suggests the efficacy
of DFS in improving UIE similar to optimally iodized salt and reflects the active supply of iodine into the system.

5.6.2.3 Impact on thyroid analytes

➢ At baseline and towards end median TSH levels were observed to be within normal range for all study groups. They also differed non significantly throughout the study period between all groups. Within group comparison for median TSH levels revealed that, towards the end E+DW (p<0.001) and E (p<0.01) groups showed significant reduction in TSH values compared to their baseline values. However, control groups did not show any significant reduction at significant level.

➢ Median FT₄ values varied significantly between groups at baseline and at the end. There was also a significant reduction in FT₄ value in each group compared to their baseline values.

➢ This in turn suggested non significant impact of DFS on thyroid analytes compared to control groups.

5.6.3 Impact on IQ and cognition

➢ Data on IQ and cognitive scores revealed that, DMT scores improved (p<0.001) significantly amongst E+DW group compared to rest of the groups, indicating better impact of iodine and iron through DFS on IQ of children than iodine only.

➢ Results on VMT also revealed improved scores for VMT in experimental (E and E+DW) groups with higher level of significance (p<0.001). However, the improvement was significant in C+DW group (p<0.001), where as non significant in C group.

➢ CT scores improved significantly in all the groups, indicating impact of NHE and improved diet on the concentration of the children, other than the familiarity factor. However, the improvement was observed to be significantly high (p<0.01) amongst E+DW group compared to rest of the group, indicating the role played by iron supplies by DFS along with deworming.

➢ Overall experimental group dominated the improved scores for all three tests.
5.6.4 Interrelation between parameters

- Interrelation between parameters revealed that, there was a significant difference between all four groups for VMT scores (p<0.01) when compared for mean change in hemoglobin 0.01->1 g/dl and also for DMT scores (p<0.05). However CT did not show any significant difference between the groups. This in turn suggests that, DMT and VMT scores are influenced by improved iron nutrition. However, CT is influenced by overall nutrition.

- When all groups were compared based on variation in UIE by raise or decrease in median UIE by 100µg/L, there was no difference observed for all the tests which were dependent on UIE.

5.6.5 Impact of supplementation, NHE and BCC on the KAP and dietary intake

- Based on the data on mean dietary intake of school children before and after NHE revealed a significant improvement amongst all age groups of the children compared to their RDA for energy, protein and fat.

- Improvement in mean dietary intake of iron was resulted due to NHE in control group, whereas NHE+DFS intake brought highly significant improvement (p<0.01) in the intake of children belonged to experimental groups.

- When the dietary intake of children was compared for before and after intervention with median percent RDA, it was observed that, there was a significant improvement in macronutrients like energy, protein and fat. However, the increase with regards to iron was highly significant (p<0.001) for experimental group, where as non significant for control groups.

- Percent distribution of RDA intake into 4 categories- <25%, 26-50%, 50-75% and >75%, revealed a significant improvement in the percentages of two later categories compared to baseline, indicating positive impact of NHE along with supplementation.

- After collecting data on FFQ at baseline, which was observed to be poor with respect to iron, iodine, Vitamin C rich and protein rich foods; post intervention data was collected only on the recommended foods.
This reflected a significant improvement in frequency of the recommended foods compared to baseline. This in turn indicated by the increased frequency for the consumption of iron rich, Vitamin C rich, protein and iodine rich foods.

There has also been a remarkable impact on the knowledge, attitude and practices of the mothers of the children upon targeted topics like iodine and iron nutrition, their importance, the storage and cooking practices for iodized salt/DFS for optimum gain of the nutrients, modified dietary practices and most cost effective modes to improve the nutritional status of their children.

Last but not the least, the acceptability of DFS was extremely high, though initially there were certain issues related to colour change, which could be sorted out and the usage of DFS could be assured for the children and their families.

CONCLUSION AND UNIQUE CONTRIBUTION AS RECOMMENDATIONS OF RESEARCH PHASE II

To conclude the present phase, validation and usefulness of double fortified salt for school aged children and adolescents which emphasize the need for consuming DFS by one and all. It has been observed to be more effective when used with other supportive strategies such as improvised micronutrient intake (iron and iodine) using DFS, improved nutrition intakes based on nutrition health education/counseling and deworming, since the children are more prone to worm infestation during their growth years.

The results of the study, viewed collectively, lead to the rejection of the hypothesis stated at base,

3. There is no significant impact of DFS supplementation (compared to control) on iron and iodine status.
4. There is no significant role of deworming in improving the efficacy of DFS (compared to controls).
5. There is no significant impact of DFS consumption on IQ/cognition test scores of the children (compared to controls).
6. There is no significant impact of NHE on nutrient intake and KAP of the subjects
Contrary to the hypothesis, the experimental group was benefited from DFS supplementation:

- Experimental group had an improvement in iron status and iodine status.
- The study population was iodine insufficient, so efforts should be targeted in achieving iodine sufficiency.
- The hemoglobin status of the population calls for an immediate action to be improved since most of the children are anemic >90%.
- Among children from rural settings, there are limited dietary sources for iron due to limited purchase power of the families and ignorance as major causes. Hence, DFS should be included in the daily diet of the families to meet partial RDA for iron and complete RDA for iodine at an economic cost and to have a sustained liberation of iron from the salt in the gut.
- Prevalence of urinary iodine insufficiency is higher (>20%) and prevalence of goitre is 12.87%. Hence, there is a need for careful monitoring of the subjects and consumption of optimally iodine fortified salt (DFS).
- Parents/ concerned family members (whenever required) should be made aware about the importance of iodine and iron nutrition and their future consequences resulted by their deficiencies during growth spurt/adolescence.
- As suggested in the phase I, it is advisable to use multiple approaches to combat micronutrient deficiencies as an initiative and support strategies from all stake holders and the government for a permanent solution.
Phase III
Upgrading salt iodization at local level and feasibility for double fortified salt production at local level

Summary

Introduction
Globally salt iodization has been proven to be one of the most renowned, workable strategy to combat iodine deficiency disorders with all its controversies. However, many of the countries have achieved the goal of reaching >90% iodization and some are still striving to achieve the same. India is one of these countries, though we had a tremendous economic growth from last decade, we have reached 71% in the year 2010 towards processing of Iodizing all edible salt respite the strict regulatory mandates from the government. Considering India’s political upheavals’, this improvement is a 20% increase from 51% reported in the year 2006.

Micronutrient malnutrition, especially iodine and iron are detrimental to the health of pregnant women and children. Therefore, it is essential to produce fortified food products which can be consumed by one and all at daily basis. Salt has been chosen to be the vehicle meeting the requirement most efficiently compared to rest of the vehicles among dietary diversified country like India. Thus, it becomes a responsibility of the salt producers to make these micronutrient reach at one go to the population striving these micronutrients.

This phase is an effort to take a step ahead towards this direction by motivating salt producers to produce DFS at local level and thereby increasing the availability and cost of DFS for a common man.

Methodology
The present research phase was undertaken with the broad objective “To upgrade the iodization among small scale salt producers and workout feasibility for DFS production”.

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Sample selection and site of the phase III

Higher prevalence of still birth and lower UIE excretion in pregnant women attending 20 PHC’s from Anand district contributed to the conceptualization of this phase. On initiating a partnership work with MI, Gujarat and UNICEF, Gujarat on upgradation of salt iodization program, N=38 producers from Anand, Nadiyad, Vadodara, Bharuch and Kheda made our study samples.

Experimental design of the Phase II

This phase was divided into 3 major sections.

Section I: Baseline salt iodization

At baseline mapping and salt sample collection was carried out from N=38 salt production units from Anand, Nadiyad, Vadodara, Bharuch and Kheda.

Specific objectives:

• To map the small scale salt units and assess the salt iodization levels in Anand, Nadiyad, Vadodara, Bharuch and Kheda districts of Gujarat.

Section II: Interventions and monitoring of iodization levels

This section included the N=34, owing to drop outs of the salt producers due to various reasons. The producers were provided with technical support and public health concern ethically by using different strategies like one to one communication at plants and conducting training-review workshops as and when required.

Specific objectives:

• To build up the capacity of small scale salt producers of these districts for salt iodization.
• To provide technical support for optimal iodization.

Post intervention data was collected on upgraded salt iodization levels in the produced salt in the production units.
Section III: Initiating concept of DFS production at local level

This section included N=3 of the selected medium and large scale salt producers. They were communicated on the role of DFS, need for technicalities, benefit: cost ratio at producer and consumer level.

- Selecting salt producers for initiation of double fortified salt at medium scale and large scale.
- To initiate the conversation on feasibility for DFS production at local level.

Feasibility trials are on progress to manufacture DFS at local levels. Three salt producers are on the move to undergo training from NIN. Initial processing and cost effectiveness of the programme have been discussed with them. Further, their convenience is awaited.

Data analysis

Simple descriptive analysis of the data was carried out. Statistical analysis was performed using Chi-square ($\chi^2$) when appropriate for categorical data. The groups were compared using ‘F’ test.

MAJOR FINDINGS OF PHASE III

- At baseline, 63.20% of the salt producers were from Anand/Nadiyad and rest were from Vadodara/Bharuch and Kheda.
- Majority of the producers were producing $\geq$5 tonnes salt/month, except 13.2% below 5 tonnes/month. Thus, the concern was more to be focused since majority of the population was consuming inadequately iodized salt.
- Baseline data revealed that, none of the districts had mean iodine content of the salt on the recommended level ($\geq$30 ppm).
- There were $>80\%$ of the units in each district which were having below 30 ppm of iodine content in the salt. This suggested lack of either technical know-how or less determination to produce quality product. Hence, the interventional strategies addressed both the issues.
- Interventional strategies included, training of the salt iodization procedure at production units, technical workshop, review workshop and subsidized supply of potassium iodate.
Towards the end of the study, the salt iodization level improved significantly (p<0.05) in Anand/Nadiyar districts and non significantly, yet improved for rest of the two districts.

In total there were 58.8% of the producers producing iodized salt at optimal level (≥30 ppm), which is a tremendous success in itself since the improvement within a span of 9 months has been 38% (baseline 20.6% to 58.8%- end).

Conceptualization of DFS has also achieved success, having N=3 of the medium and large scale salt producers being eager to become a part of our efforts towards establishing DFS (NIN formula) production at local level.

To overcome the need as a participatory approach National Institute of Nutrition was approached for facilitating the premix supply and technology transfer. Discussions for training the producers and technology transfer have been initiated. Further procedures are being addressed. The work is in progressive state.

**CONCLUSION AND UNIQUE CONTRIBUTION AS RECOMMENDATIONS OF RESEARCH PHASE III**

It can be stated that advocacy effort provided towards technical salt iodization, and availing concern from the salt producers for double fortified salt production at local level could be achieved successfully.

The results of the study phase, viewed collectively, lead to the rejection of the hypothesis stated at base indicated the following:

1. There will be no significant impact of advocacy measures on achieving optimal salt iodization by the small and medium scale salt units.
2. It is not feasible to achieve production of DFS at local level.

Contrary to the hypothesis, the results of the study phase revealed that,

- Majority of the producers have achieved recommended levels of salt iodization at production level (30 ppm) (60%).
- Majority of the producers have started putting their conscious efforts and are willing to produce quality product.
- Three of the producers are eager to produce DFS at local level, undergo technical training and salt unit upgradation as an initiative towards DFS production.
RECOMMENDATIONS

✓ Double fortified salt should be implemented as nationwide strategy to combat two essential micronutrient deficiencies among all age groups.

✓ Screening for thyroid dysfunction and iodine deficiency should be implemented as target coverage under government programmes for safe pregnancy.

✓ Nutrition health education and campaigning should be carried out at massive scale to create awareness amongst general public regarding micronutrient deficiencies, their consequences for all age groups at various life stages and strategies to combat these deficiencies.

✓ Deworming should be implemented as compulsory procedure for school curriculums under the government schemes for all school aged children and adolescents.

✓ Salt producers should be advocated and motivated to achieve optimal salt iodization with apt technical proficiency.

✓ Technical training, financial and plant upgradation support-machineries (subsidized cost) should be provided by the government to the salt producers who are willing to initiate DFS production at their units.

RECOMMENDATIONS FOR FUTURE RESEARCH

✓ DFS has been proven efficacious enough to improve iron and iodine status of the study population- pregnant women and school aged children. However, due to various limitations, the study was conducted for a stipulated time period, population and region. Thus, long term supplementation and its impact need to be studied on larger sample size and on regional groups. To sum up there is a need to carry out multicentric studies by the government agencies including nutrition institutions to assess the blanket impact of DFS consumption.

✓ School children and adolescents are very sensitive, rather indicative group for assessing micronutrient deficiencies. Thus, longitudinal studies including adolescent
girls supplemented with DFS, their pregnancy and birth outcome could be one of the key research to observe the contribution of iron present in DFS when consumed for a long term.