

Research Article

Double fortified salt: an effective measure to control micronutrient deficiencies in Indian pregnant women

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ABSTRACT

Background: Pregnant women and children are extremely affected by micronutrient deficiencies with reference to fetal growth manifestations as well as post birth. Iodine and iron deficiency leads to poor life quality during early fetal development and young children may compromise on their IQ and cognitive development. It is reported that there is an interrelations between iron and iodine metabolism. However, references on using double fortified salt (DFS) as a tool to improve micronutrient status among pregnant women are limited; hence this study was designed.

Methods: Pregnant women (n=150) were enrolled from a semi government hospital of urban Vadodara, Gujarat, India during first trimester (<12 weeks) and followed up till the end of gestation, n=75 were divided in experimental (DFS supplemented) and control (IS consumers) group. Impact on iron and iodine status was assessed by Hb concentration and UIE respectively.

Results: Mean Hb improved significantly ($p<0.001$) (+0.42 g/dl) in experimental group and reduced insignificantly (0.20 g/dl) in control group at the end, since DFS provided additional ~93 mg of iron within 6 months of supplementation. Median UIE improved insignificantly (278.6 to 299.01 µg/L) in experimental group and decreased significantly ($p<0.05$) (376.59 to 288.66 µg/L) in control group.

Conclusions: Hence, we conclude that DFS could improve iron and iodine status of experimental group compared to control group. It is an effective measure to control two essential micronutrient deficiencies together.

Keywords: Double fortified salt supplementation, Anemia, Pregnancy, Iodine deficiency, Iron

INTRODUCTION

Micronutrient malnutrition affects pregnant women and children extremely with reference to growth manifestations in gestation as well as after birth. Of the various micronutrients, iodine and iron are the utmost essential ones. 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.¹ Recent reviews from India suggests that the prevalence of iodine deficiency (ID) (UIE<150 µg/dl) ranges from 30-95% among pregnant women which suggests comparatively higher prevalence of iodine deficiency than the developed countries.²⁻¹⁰ However, iron plays a vital physiological role in growth, development, metabolic reactions, cofactor for a number of enzymatic activities and cognitive development of the fetus. Untreated chronic/severe maternal iron deficiency

anemia (IDA) may prove to be detrimental to the developing fetus. The prevalence of anemia amongst Indian pregnant women is more than 60%.¹¹ WHO estimates that even among the South Asian countries, India has the highest prevalence of anemia and it also contributes to about 80% of the maternal deaths due to anaemia.¹² Early fetal development affected by iodine and iron deficiency leads to poor life quality. The shortage of these essential micronutrients, its duration and severity may alter the outcome. In developing countries where the prevalence of these deficiencies is still considerably high, social, and economic development are also likely to be affected.¹³

Globally, researchers have found interesting interrelations between iron and iodine. A study among pregnant women with mild iodine deficiency suggested that poor maternal iron status predicts higher TSH and lower TT₄ concentrations.¹⁴ A central mechanism in this effect is impairment of the heme-dependent enzyme TPO, limiting synthesis of thyroid hormone, and reducing circulating TT₃ and TT₄.¹⁵ In young anemic women, treatment with iron may increase circulating thyroid hormone concentration.^{16,17} These studies and many others suggest a strong need for fortified food products to provide iodine and iron together.

Various studies have showed improvement in iodine and iron status using number of fortified food items like instant noodles, sugar, fish sauce, margarine, biscuits, seasoning powder and beverage powder.¹⁸⁻²³ In addition to that, double fortified salt (DFS) has emerged as an effective measure to target ID and IDA in under privileged provinces of different countries as well as in countries with dietary diversification like India. Considering the physiological properties of iodine as well as iron and the environment of common salt as a vehicle, the levels of iodine and iron are intended to be for preventive purpose and not for therapeutic purpose. Iron in DFS will meet about 30% of recommended daily intake only.²⁴ However, this increase in iron intake still expected to be beneficial during pregnancy, adolescence and in anemic subjects since the absorption.

METHODS

Study population

Pregnant women (n=150) during first trimester (<12 weeks) were enrolled and followed up till the end of gestation from an antenatal clinic of semi government hospital of urban Vadodara, Gujarat, India (October 2009 to November 2010).

Women with singleton pregnancy were selected and were randomly divided into experimental (n=75) and control (n=75) group using a computer generated list. Minimum three visits were required by the pregnant women (1st, 2nd and 3rd trimester). However, due to various reasons (abortion, miscarriages, missing follow-ups,

unwillingness to participate and non-reachability) n=121 (n=67-experimental group and n=54- control group) could complete the study.

Data collection

All the pregnant women were explained the purpose of the study and signed written consent were collected (in local language). Further, background information, obstetric history, socioeconomic status, and anthropometric parameters (height and weight) were recorded. During 2nd trimester and 3rd trimester, the weights were recorded to observe the total weight gain during gestation. They were later compared with standard guidelines.²⁶ Nutrient intake was recorded using 24 hr dietary recall information. In case of unusual intakes, it was recorded for last two days and then the average was calculated.

DFS production and procurement

Double fortified salt was manufactured at Ankur Chem food Pvt Ltd., Gandhidham, Gujarat. It was produced on a ribbon blender following the standard technology and formula transferred by National Institute of Nutrition (NIN). It was procured in 3 batches of 500-1000 kgs. It contained edible common salt (NaCl: >98.0%, Mg: <0.1% and moisture: <1.5%), Sodium Hexa Meta Phosphate (SHMP): 1%, FeSO₄.7H₂O: 0.50% (Fe = 1000 ppm) and KIO₃: 0.0067% (I = 40 ppm). The homogeneity of iodine and iron content in the salt was established at the manufacturing stage by assessing the micronutrient contents using a method established by BIS.²⁷

DFS supplementation

Women in experimental group were supplemented with DFS as a replacement to their iodized salt where as women in control group were encouraged to consume iodized salt since the availability of the adequately iodized salt (>15 ppm) among the collected household salt samples was 83%. DFS was packed in 1 kg packets and experimental group were supplied these packets based on the monthly family need to improve the compliance among the subjects. Each house received 1-2 kgs of salt/month.

Sample collection, storages and methods of estimation

Urine (10-50 ml) and blood samples (500 µl) were drawn from the subjects at the time of enrollment and at the end of each trimester to assess their iodine (urinary iodine excretion µg/L) and iron status (hemoglobin g/dl); urine samples were stored at 25°C and 2 ml of toluene was added as preservative to each sample. Urinary iodine excretion (UIE) was measured (at 405 nm) using modified simple microplate method using ELIZA reader (TecanAutria GMBH, Europe) at ICCIDD Laboratory, Delhi. Venous blood samples were collected for

hemoglobin estimation using cyan met-hemoglobin method.²⁸ The hemoglobin (Hb) was assessed on the samples within few hours of blood collection and was read on spectrophotometer (spectronic 20D) at 304 nm.

Standard classifications

IDA was defined using UNICEF/UNU/WHO criteria for hb concentration (g/dl) during pregnancy (≥ 11 : normal, 10.0-10.99 g/dl: mildly deficient, 7.0-9.9 g/dl: moderately deficient and < 7 g/dl: severe deficient).²⁹

ID was defined using WHO criteria for UIC during pregnancy (< 150 : inadequate, 150-249: adequate, 250-499: above requirement, ≥ 500 : excess).¹

Statistical analysis

The data was processed, entered and analysed in the statistical package for social sciences for windows version 15.00 (SPSS 15.0). Basic analysis was carried out using Chi-square (X^2) as and when appropriate for categorical data. Results of present study are reported as mean ($\pm$ sd). UIE pattern was reported as median. For comparing data between all three trimesters one way ANOVA, along with Bonferroni post-hoc analysis was carried out. Further, to compare data between paired samples, paired 't' test was used; 25th and 75th percentile was calculated to reveal $\pm 2SD$ from the mean/median values. A two-tailed p value < 0.05 was considered statistically significant.

Ethical statement

This study was conducted according to the guidelines laid down in the declaration of Helsinki and all procedures involving human subjects/patients were approved by the ethical committee of the home institution ethical board in compliance with the guidelines issued by Indian council of Medical research (No. F. C. Sc FN ME70). Written permission from the hospital authorities and concerned doctors was availed before the commencement of the study. All pregnant women were on iron folic acid and calcium supplements during 2nd and 3rd trimesters as per the government strategy, the impact of DFS surplus to the IFA consumption was measured among experimental group compared to control group. Compliance rate for IFA consumption was recorded. Subjects belonged to both the groups were provided nutrition health education towards general awareness regarding iodine and iron nutrition during pregnancy and impact on neonatal outcome.

RESULTS

All the pregnant women belonged to lower or lower middle socioeconomic group. Mean age, height and BMI were 23 (± 3) years, 151 (± 5.36) cms and 19.90 (± 3.18) kg/m² respectively. Mean BMI was falling under normal category as per standard guidelines (IOM 2009). Mean

weight gain during gestation was 5.06 (± 3.09) kg [45.38 (± 7.91) (initial) – 50.45 (± 8) (final)]. Mean gestational age was 38 (± 2) weeks.

Impact on iron status

After six months of supplementation, there was 1.5% increase [10.4 (initial) - 11.9 (final)] in iron sufficient subjects and 10.5% [20.9 (initial) - 10.4 (final)] reduction in moderately anemic subjects who shifted to mild or normal category in experimental group (Figure 1A). In control group, with progression of gestation, 11.1% [14.8 (initial) - 3.7 (final)] normal subjects shifted to anemic category. Average 70% of control group women remained mildly anemic throughout gestation and more than 20% remained moderately anemic (Figure 1B) and there was a gradual reduction in percentages of normal subjects till the end.

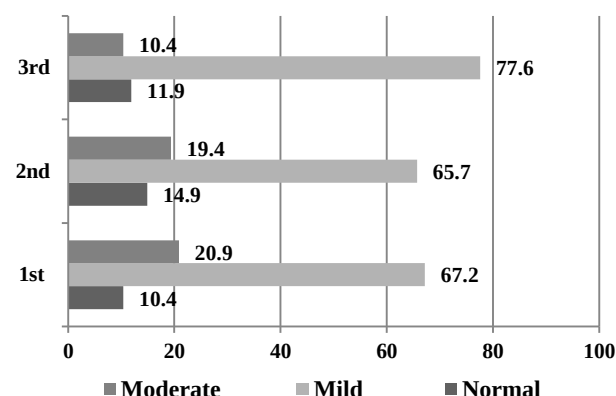


Figure 1A: Anemia prevalence in experimental group.

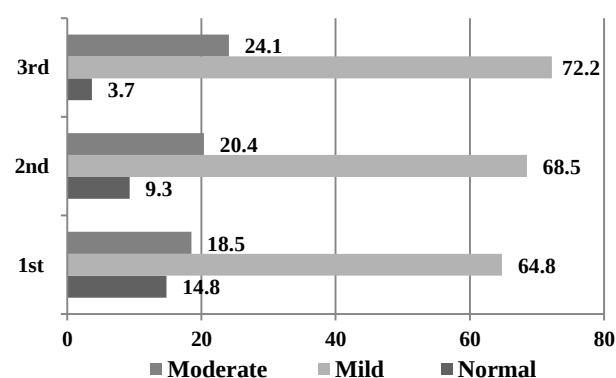


Figure 1B: Anemia prevalence in control group.

There was no significant difference in mean Hb between both the groups at the time of enrollment. In experimental group, mean Hb 0.42 g/dl [9.44 (initial) – 9.86 (final)] increased significantly ($p < 0.001$) towards the end. However, in control group, it decreased significantly ($p < 0.05$) during 2nd trimester (9.00 g/dl) and did not improve significantly towards the end (9.15 g/dl) (Table 1). During 3rd trimester results showed significant difference between both the groups (0.71 g/dl) ($p < 0.001$).

Table 1: Mean hemoglobin concentration of both groups.

		Hemoglobin levels (g/dl)		
		Mean (25 th - 75 th percentile)		
	N	1 st trimester	2 nd trimester	3 rd trimester
Experimental	67	9.44 (9.50-10.00)	9.18 ^{NS} (8.50-10.00)	9.86 ^{a,b} (9.50-10.50)
Control	54	9.35 (9.00-10.00)	9.00 ^c (8.50-10.00)	9.15 ^d (8.87-10.00)

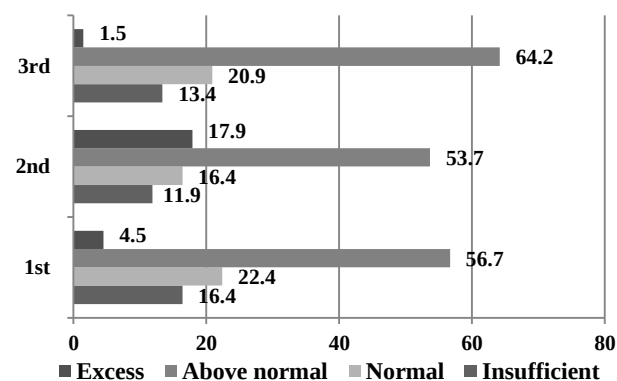
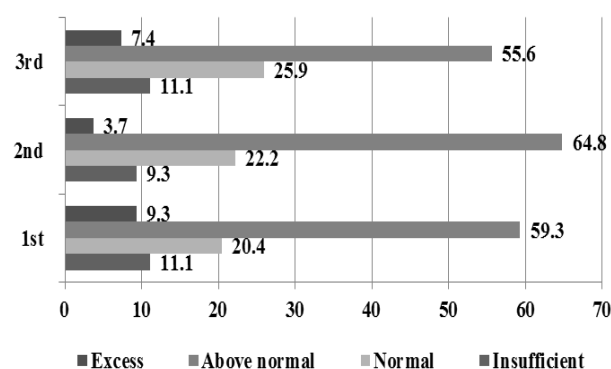
^a Values differed significantly ($p<0.001$) between 3rd trimester and 1st trimester in experimental group; ^b Values differed significantly ($p<0.001$) between 3rd trimester and 2nd trimester in experimental group; ^c Values differed significantly ($p<0.05$) between 1st trimester and 2nd trimester in control group; ^d Values differed significantly ($p<0.001$) between experimental and control group at 3rd trimester.

Impact on dietary intake and iron availability

Initial - final energy, protein and fat intake of the participants were 1251 (± 350) - 1539 (± 412) k. cal, 24 (± 9) - 32 (± 11) gm and 37 (± 12) - 41 (± 14) gm respectively. The increase in intake was significant ($p<0.05$) for all these nutrients.

It was important to note that, both the groups were on iron-folic acid (IFA) (60 mg elemental iron) supplements under the government scheme, minimum for 120 days. Hence, the availability of iron from IFA tablets can be calculated to approximately 7200 mg within 4 months and it could be 1800 mg per 6 months from DFS intake (Table 2). Review suggests that the absorption rate of iron from IFA is 8% and the average compliance to IFA was 78% in our study.³⁰ In case of DFS, the reported rate of iron absorption in rice based population was 6.1% whereas it may vary in our population since they were on wheat-rice-bajra based diet.³¹ However, the data was

calculated at 4% since there is no data available on in vitro bioavailability of iron from wheat based diet. The approximate calculation (Table 2) suggests that the experimental group received 1800 mg additional and 93 mg bio-available iron within 180 days of supplementation and in addition to ~447 mg bioavailable iron from IFA supplements compared to control group. This can be one of the major reasons towards significant improvement in Hb conc. compared to control group.

**Figure 2A: UIE classification in experimental group.****Figure 2B: UIE classification in control group.****Table 2: Approximate intake, absorption and availability of iron.**

Group	Iron source	Dose	Duration	Availability	Compliance	Absorption
Experimental	IFA	60 mg/day	120 days	7200 mg	77.6%= 5587.2 mg	8%= 446.97 mg
	DFS	10 mg/day	180 days	1800 mg	95.5%=1719 mg	4%=93 mg
	Total					551.82 mg
Control	IFA	60 mg/day	120 days	7200 mg	79.6%=5731.2 mg	8%= 458.49 mg
	Total					458.49 mg

Data from 24 hr iron intake showed that all the pregnant women consumed more than 80% iron deficient diet compared to standard RDA.³² The initial recorded iron intake was 5-6 mg/day which improved by 30% [15.6 (initial) - 46.16 (final)] ($p<0.001$) after DFS

supplementation in experimental group and remained almost unchanged among control group. This calculation on total iron intake was derived from the daily dietary iron and DFS intake of the experimental group, which was observed to be approximately 10 gm per day.

Impact on iodine status

Initial UIE reported 86% women with sufficient levels. Till the end of gestation, iodine insufficiency reduced by 3% [16.4 (initial) - 13.4 (final)] (Figure 2A) in experimental and unchanged in control group (Figure 2B).

Since both groups were consuming sufficiently iodized salt, the median UI difference was insignificant for all three trimesters. Median UIE was 278.60 and 376.59 µg/l respectively in experimental and control group at baseline. UIE remained higher in 2nd trimester (347.60 µg/l) ($p < 0.05$) and 3rd trimester (299 µg/l) compared to 1st trimester in experimental group. However, it decreased in 2nd (314.68 µg/l) and 3rd trimester (288.66 µg/l) ($p < 0.05$) in control group. Median UIE varied insignificantly between both the groups at each time point (Table 3).

Table 3: Median UIE levels of both groups.

Urinary iodine excretion (µg/L) Median (25 th - 75 th percentile)			1 st trimester	2 nd trimester	3 rd trimester
	N				
Experimental	67		278.60 (105.30-489.740)	347.60 ^a (123.56-960.50)	299.01 ^b (121.37-492.70)
Control	54		376.59 (131.95-918.01)	314.68 (116.94-492.19)	288.66 ^c (106.61-783.00)

^a Values differed significantly ($p < 0.001$) between 2nd and 1st trimester in experimental group; ^b Values differed significantly ($p < 0.001$) between 3rd and 2nd trimester in experimental group; ^c Values differed significantly ($p < 0.05$) between 1st and 3rd trimester in control group.

DISCUSSION

Impact on iron status

In India, the only data available on NIN-DFS supplementation amongst pregnant women revealed no difference in hemoglobin levels between experimental and control group after 2 years of supplementation in tribal villages of Andhra Pradesh, though there was a significant rise in Hb concentration (0.8g/dl) in experimental group ($p < 0.01$).²⁵ This population was on rice based diet. However, in our study a significant difference was observed between both the groups at the end. Our population was on wheat-rice-bajra based diet. Another study conducted by NIN in 4 centers of India on effect of iron fortified salt supplementation on the hemoglobin concentration included pregnant women as subjects but separate data of pregnant women has not been presented.³³ Thus, our study could be an important base to the further research on DFS efficacy trial in pregnancy. During 1st trimester, there is nominal increase in iron requirement, which can be met by the cessation of menses.³⁴ However, when maternal blood volume

expands and the fetus grows the need for iron rises with the progression in pregnancy. The rate of iron absorption varies accordingly. Iron balance in pregnancy can be maintained only when there are adequate stores (need to be ≈ 300 mg). In general, a total iron requirement of a 55 kg healthy woman is approximately 1000 mg. converting it to the daily needs, the requirement is 0.8 mg during 1st trimester, between 4 to 5 mg during 2nd trimester and 6 mg during third trimester.³⁵ However, more iron stores will be required if the diet is with suboptimal iron levels. In our study subjects, who belonged to lower strata of community- irrespective of urban or rural localities, the dietary pattern was far away from meeting the iron requirement during normal conditions and thus leading to obvious dietary deficit during pregnancy. The lowest Hb conc. was observed at 2nd trimester owing to the hemodilution and increased iron requirement, suggesting that the pregnant women entered pregnancy with lower iron stores and the consumption of iron rich foods was still lower. Hence, there was a need for supplementation (DFS) from the adolescence to the end of gestation to bring about visible change in iron content of their diet. Our results also revealed that $>80\%$ of the pregnant women were iron deficient when they became pregnant and mean hemoglobin content of both the groups remained under moderately iron deficient category for all three trimester. However, during 3rd trimester there was a significant increase in Hb conc. of experimental group (0.42 g/dl) compared to non-significant decrease in control group (0.20 g/dl). This suggests that iron supplements and DFS may play a major role in improving iron status of the pregnant women as two effective strategies. However, at this stage compliance to iron folic acid supplements becomes a major concern, since majority of the pregnant women who receives IFA supplement from government hospital shows lower adherence to the medications provided free of cost.³⁰

As per the calculation provided in Table 2, the rate of absorption and compliance to the supplements plays vital role towards improving hemoglobin concentration amongst the subjects. Our results have clearly shown additional 93mg bioavailable iron through DFS in the diet of supplemented group and thus could be the major contributor towards improving Hb concentration among experimental group compared to control group. DFS supplementation could also improve dietary iron intake of experimental group through their daily diet other than their IFA supplements and the compliance rate was $>90\%$ which is a great success in itself. From our results there is a clear cut demarcation that, the experimental group improved in their iron sufficiency status by 1.5%. This is indicative that, there was a sufficient circulating pool of iron available for the mother and baby to combat with their daily physiological needs. This could be attributed to sustained release of iron from DFS. Owing to the simultaneous reduction in non-anemic subjects in control group, our results reflect the role and contribution of DFS as an additional strategy on improving maternal Hb conc. along with IFA supplementation.

Impact on iodine status

Pregnancy is a stage where all the essential nutrients/micronutrients have their vital role to play towards fetal development and their requirements varies compared to non-pregnant adults. Iodine requirement during pregnancy increases due to mainly three reasons including: an increased requirement for thyroxine (T4) in order to maintain normal metabolism in the mother; a transfer of T4 from the mother to the fetus; and a supposed greater than normal loss of iodine through the kidneys due to an increase in the renal clearance of iodide. Hence, the RDA for pregnant women has been increased to 250-300 mcg/day compared to non-pregnant adults and adolescents.¹ In healthy adults the absorption rate of iodine is >90%. Thus, the daily iodine intakes for pregnancy (250 mcg) would correspond to UI app. 185-215 mcg/l.³⁶ In healthy adults the absorption rate of iodine is >90%.

Optimally iodized salt (recommended 150 ppm) can meet this requirement if consumed 10gm/day. In our study, mean iodine content of DFS was 40 ppm (recommended 40 ppm) and iron at 1050 ppm (recommended 1000 ppm) at the time of production. The stability of DFS was also assessed after 1 year and the contents remained closer to the recommended levels for iodine (37.5 ppm) and iron (979 ppm). Thus, it suggests that DFS could meet 100% iodine RDA for pregnant women whereas iodized salt may or may not. It was apparently more stable and provided more iodine compared to iodized salt. The impact was observed on UI content also where median UI in experimental group increased during 2nd and 3rd trimester compared to 1st trimester whereas there was a gradual reduction in UI in control group with the progression of gestation. On contrary, a study reported no significant difference in UI concentration between three trimesters of pregnancy in women residing in iodine sufficient region.³⁷

In our study situation where optimally iodized salt is available in the region, we observed non-significant difference in UI conc. between experimental and control group at any trimester. Further, studies on DFS supplementation in all over the world have also reported that DFS supplemented group did not show significant difference compared to iodized salt group.³⁸⁻⁴¹

CONCLUSION

DFS was proved to be potential iron intervention strategy towards improving iron and iodine status of the supplemented group when compared to control group. It acted as an additional benefit to current optimal iodization program without enhancing monthly grocery budget, efforts towards different method of cooking and showed great acceptability of the population. Hence, it is recommended that, the DFS should be made available through various existing, well-functioning government health schemes in India, like mid-day meal, integrated

child development services programs and anganwadi for the most vulnerable section of the population i.e. pregnant women, children and adolescents. It should be incorporated into the public food distribution systems and in local market to reach majority of the population. We also recommend that large scale studies to be carried out considering our findings as pilot study and *in vitro* bioavailability of iron using DFS in various food preparations should be carried out.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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