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**Original Research Article**

# Impact of stopping the use of iron fortified iodised salt on Hb levels

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## ABSTRACT

**Background:** Research studies have shown that use of iron fortified iodised salt (DFS) improved Hb in women, children and men. There are no publications on impact of stopping DFS on Hb levels.
**Methods:** A community-based randomised study to assess the impact of DFS use on Hb was being carried out in Delhi. Between April-September 2020 salt could not be supplied and families were requested to purchase iodised salt (IS) from the market and use it. Blood samples from persons from these families were collected to assess impact of not using DFS for 6 months on Hb.
**Results:** Comparison of mean Hb after 12 months use of the allocated salt and Hb after six-month use of IS by all three groups showed that: (1) there was a small reduction in Hb in all three groups after using IS for 6 months; fall was statistically significant in women in group 3; (2) women who had earlier used DFS had higher mean Hb and ferritin as compared to those who had used IS, both after 12 months of use of DFS and after 6 months of use of IS; (3) changes in ferritin varied and were not consistent.
**Conclusions:** DFS users continued to have a higher mean Hb as compared to IS users even after discontinuing use of DFS for 6 months. The impact of this higher Hb when they restart using DFS for further 12 months has to be investigated.

**Keywords:** CRP, DFS, Ferritin, Haemoglobin, Iodised salt, Stopping DFS use

## INTRODUCTION

Prevalence of anaemia in India is high across all age, sex and physiological groups.[1,2] Iron deficiency is mainly due to low dietary intake of iron and poor bio-availability of iron from Indian diets.[3] Anaemia Mukt Bharat initiative recommended multi-pronged interventions to improve Hb levels: nutrition education to increase vegetable intake, use of iron-fortified food stuffs to increase iron intake without any dietary modification, iron and folic acid supplementation to vulnerable groups and 'test and treat' strategy for management of anaemia.[4] Currently India is the number one country in the world in vegetable production but per capita vegetable intake continues to be low partly because of problems in availability and high cost. Iron folic acid (IFA) tablet supplementation to women, children and adolescent girls are provided under the programme but coverage is low and compliance is poor.

In India, fortification of salt with iron has been shown to be a feasible, affordable and sustainable method of bridging the gap in iron intake and improving Hb status.[5] Food Safety and Standards Organization of India (FSSAI) has approved two formulations of iron and iodine fortified salt (double fortified salt- DFS).[6] Research studies in India and elsewhere have shown that when DFS has been consumed for 6 to 12 months there was improvement in Hb and ferritin.[7-10] Government of India's guidelines recommend use of DFS in preparing hot cooked meals for children under the school midday meal (MDM) programme and integrated child

development services (ICDS).[11] Some states have provided DFS through public distribution system (PDS) and through open market. In a vast country like India there are difficulties in ensuring continued supply of DFS to ICDS and MDM programmes or through PDS, especially in the initial phases of the programme. There had so far not been any publications documenting the impact of stopping the use of DFS on Hb levels. This might be partly due to the fact that it was considered unethical to stop DFS use to study impact of discontinuation of DFS use on Hb.

Our institution was conducting a community based, three-armed open randomised study of two formulations of DFS and iodised salt (control arm) to assess the impact use of DFS for 18 months on Hb and ferrtin.[12] The study had to be terminated after 12 months because on March 24th 2020, Government of India imposed a rigorous lockdown to delay the COVID pandemic. The disruption in supplying DFS for 6 months provided us an opportunity to assess the impact of stopping the DFS use on Hb levels. When the travel restrictions were eased in October 2020, families still residing in the area were contacted. Blood samples were collected from available and willing persons from these families to assess impact of not using DFS for six months on Hb and ferritin.

## METHODS

A community based open randomized three arm study (one arm IS and two arms two formulations of DFS) to assess the impact of use of DFS on Hb status was conducted in three purposively chosen blocks in South Delhi where our institution has been working for the past decade. From the census of households in the area the first 750 families who fulfilled the inclusion criteria and consented to participate in the study were allocated to one of the three groups [250 each in group 1 (IS), group 2 (DFS ferrous sulphate DFS FS), and group 3 (DFS ferrous fumarate DFS FF)] using a computer-generated random allocation. The families had completed 12 months use of IS or either one of the DFS as per the random allocation when Government of India imposed a strict lockdown to delay the spread of COVID-19 epidemic.[12] Many migrant families returned to their villages and were lost for further follow up. Between April to September 2020 DFS could not be supplied even to the families who remained in Delhi due to transport restrictions. The research team kept in touch with the families telephonically and advised them to purchase iodised salt (IS) from the market and use it. In October 2020, the research team went back to the community and contacted the families who were available in the area and explained to them the importance of obtaining information on the impact if any of discontinuing the use of DFS on Hb and ferritin levels. Informed consent was obtained from family members who were available and willing and blood samples were collected.

Hb estimations by cyanmethaemoglobin method were carried out at our institution on the day the samples were collected; technicians who undertook Hb estimation were blinded to the group from which the samples were drawn. All samples were coded and processed samples were stored in deep freeze until analysis. Ferritin assay was done by electrochemiluminescence immunoassay (ECLIA) and CRP estimation was done using enhanced immunoturbidimetry assay using COBAS 6000 in a laboratory certified by the National Accreditation Board for Testing and Calibrating of Laboratories, India. The laboratory fared well in the external quality assurance programmes; internal quality assurance tests showed that there was excellent concordance in measurement of parameters between coded duplicate samples. Technicians who undertook ferritin and CRP assays were given coded samples and did not know the group from which the samples were collected.

### *Data entry, data cleaning and data analysis*

Data entry was done in MS excel; data cleaning was done using MS excel and SPSS. Data analysis was done with SPSS version 27 and Stata version 15.

Mean Hb, ferritin and CRP levels at enrolment and after use of the salt provided by research team to each of the three groups for 12 months were compared with the Hb, ferritin and CRP levels in the three groups after 6 months of use of iodised salt. Inter-group comparisons of Hb, ferritin and CRP were made at enrolment, 12 months of use of the allocated salt and after six months of use of iodised salt. Statistical significance was assessed using t test. P values $<0.05$ were considered statistically significant.

## RESULTS

Analysis of data on the socio-demographic profile of the study families remaining in the study area, showed that there were very few migrant families. Majority of the women were between 20 and 30 years of age; they were literate home makers. Majority of men were school or college educated, were semi-skilled or skilled workers. They had experienced job loss/wage reduction during the lockdown. Families had curtailed all inessential expenditure and tried to cope with the financial constraints. They had utilized the PDS supply of the food grains as well as hot cooked food provided free of cost during lockdown. Their access to health care especially antenatal care and immunization for children were disrupted during lockdown. When the research team went back to the families, the families readily agreed to continue receiving the salts as per allocation and participating in the study.

The number of women, men and children available and willing from whom blood samples were collected at enrolment (0 month), after use of allocated salt provided by research team for 12 months under randomized trial

(RT) and after all families used only IS for six months during the lockdown (LD) is given in Table 1.

**Table 1: Community based randomised studies on impact of DFS: persons enrolled and followed up.**

| | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
|---|---|---|---|---|
| **Women** | Enrolment | 319 | 298 | 297 |
| | 12 months | 122 | 122 | 116 |
| | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | 6 months | 139 | 101 | 121 |
| **Men** | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
| | Enrolment | 122 | 101 | 100 |
| | 12 months | 54 | 35 | 48 |
| | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | 6 months | 36 | 27 | 29 |
| **Children** | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
| | Enrolment | 85 | 60 | 63 |
| | 12 months | 67 | 43 | 52 |
| | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | 6 months | 13 | 12 | 17 |

**Table 2: Changes in Hb, ferritin and CRP after 12-month of use of salt as per random allocation and use of purchased IS for 6 months by all the three groups.**

| | | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
|---|---|---|---|---|---|
| **Hb (gm/dl)** | Women | 12 months | 11.0±1.32 (122) | 11.4±1.38 (122) | 11.2±1.34 (116) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 10.8±1.55 (100) | 10.8±1.55 (127) | 11.0±1.33 (125) |
| | Men | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
| | | 12 months | 13.2±1.22 (54) | 13.5±1.48 (35) | 13.2±1.40 (48) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 13.1±1.40 (28) | 13.3±1.26 (29) | 13.6±1.51 (31) |
| **Ferritin (ng/ml)** | Women | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
| | | 12 months | 32.8±32.144 (107) | 39.1±32.89 (104) | 41.3±41.23 (106) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 41.7±38.57 (94) | 37.7±37.30 (124) | 41.6±40.86 (120) |
| | Men | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
| | | 12 months | 105.2±60.22 (48) | 99.0±55.80 (31) | 124.4±59.94 (44) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 114.4±49.20 (27) | 130.6±57.57 (28) | 110.6±69.05 (31) |
| **CRP (mg/L)** | Women | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
| | | 12 months | 3.3±4.56 (107) | 3.0±3.40 (104) | 3.3±3.57 (106) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 3.7±3.73 (94) | 3.1±3.56 (123) | 3.3±3.65 (120) |
| | Men | RT | Gr 1 IS | Gr 2 DFS FS | Gr 3 DFS FF |
| | | 12 months | 2.1±3.10 (48) | 2.1±3.14 (31) | 3.3±3.31 (44) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 2.3±2.65 (27) | 2.9±3.90 (28) | 3.3±3.35 (31) |

Differences over time (t test): RT 12 months and LD 6 months: Fall in Hb in women in Gr 2 p value <0.001; increase in ferritin in men in Gr 2 p value 0.04. Inter-group differences (t test): RT 12 months Hb in women between Gr 1 and Gr 2: p value 0.02.

As schools were closed in 2020, children from many families went to villages and numbers of samples from children using IS for six months were low. Therefore, data analysis was carried out only on samples collected from men and women (Table 1).

Changes in mean Hb, ferritin and CRP after 12 months of use of salt as per random allocation and use of purchased IS for 6 months during lockdown by all the three groups in all samples is given in Table 2.

**Table 3: Changes in Hb after 12-month of use of salt as per random allocation and use of purchased IS for 6 months by all the three groups (paired samples).**

| | | RT | IS | DFS FS | DFS FF |
|---|---|---|---|---|---|
| **Hb (gm/dl)** | Women | 12 months | 10.9±1.21 (63) | 11.2±1.43 (61) | 11.3±1.30 (66) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 10.7±1.35 (63) | 11.1±1.54 (61) | 11.2±1.51 (66) |
| | Men | RT | IS | DFS -FS | DFS FF |
| | | 12 months | 13.2±1.11 (14) | 13.7±1.02 (7) | 13.0±1.07 (10) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 13.6±1.21 (14) | 13.0±1.04 (7) | 13.3 ±0.72 (10) |
| **Ferritin (ng/ml)** | Women | RT | IS | DFS FS | DFS FF |
| | | 12 months | 31.7±33.44 (60) | 39.8±36.34 (53) | 39.7±35.23 (59) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 26.7±20.78 (60) | 41.7±43.88 (53) | 41.6±37.08 (59) |
| | Men | RT | IS | DFS FS | DFS FF |
| | | 12 months | 121.9±58.50 (12) | 113.1±52.26 (7) | 147.2±54.61 (9) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 116.0±52.99 (12) | 129.7±68.22 (7) | 135.2±55.81 (9) |

Differences over time (paired t test): RT 12 months and LD 6 months: None of the differences in Hb or ferritin in men or women were significant. Inter-group differences (t test): LD 6 months ferritin in women Gr 2 versus Gr 1 p value 0.02; Gr 3 versus Gr 1 p value. 0.008

**Table 4: Changes in Hb in anaemic and non-anaemic women after 12-month of use of salt as per random allocation and use of purchased IS for 6 months by all the three groups (paired samples).**

| | | RT | IS | DFS FS | DFS FF |
|---|---|---|---|---|---|
| **Hb (gm/dl)** | Anaemic women | 12 months | 10.6±0.98 (54) | 10.5±1.09 (43) | 10.8±1.07 (50) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 10.5±1.28 (54) | 10.6±1.41 (43) | 10.8±1.51 (50) |
| | Non-anaemic women | RT | IS | DFS FS | DFS FF |
| | | 12 months | 12.8±0.57 (9) | 12.8±0.55 (18) | 12.8±0.63 (16) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 12.1±0.87 (9) | 12.4±0.92 (18) | 12.2±0.98 (16) |

Differences over time (paired t test) and Inter-group differences (t test) None of the differences were statistically significant.

**Table 5: Changes in Hb at enrolment and use of purchased IS for 6 months by all the three groups (paired samples).**

| | | RT | IS | DFS FS | DFS FF |
|---|---|---|---|---|---|
| **Hb (gm/dl)** | Women | Enrolment | 10.4±1.57 (129) | 10.7±1.58 (92) | 10.6±1.68 (107) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 10.8±1.42 (129) | 11.1±1.59 (92) | 10.9±1.49 (107) |
| | Men | RT | IS | DFS FS | DFS FF |
| | | Enrolment | 13.3±1.23 (25) | 13.7±1.13 (10) | 13.2±0.91 (16) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 13.4±1.51 (25) | 13.2±1.26 (10) | 13.1±0.93 (16) |
| **Ferritin (ng/ml)** | Women | RT | IS | DFS FS | DFS FF |
| | | Enrolment | 32.2±33.50 (120) | 27.5±25.96 (88) | 28.8±34.64 (98) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 38.7±37.81 (120) | 35.8±33.24 (88) | 44.6±41.21(98) |
| | Men | RT | IS | DFS FS | DFS FF |
| | | Enrolment | 101.4±65.95 (19) | 88.9±49.26 (10) | 128.5±56.21(15) |
| | | LD | Gr 1 used IS | Gr 2 used IS | Gr 3 used IS |
| | | 6 months | 102.6±60.03 (19) | 125.5±56.13 (10) | 152.6±60.81 (15) |

Differences over time (paired t test): Enrolment and LD 6 months. Hb in women Gr 1 p=0.03; ferritin in women Gr 3 p value 0.004. Inter-group differences (t test): At LD 6 months ferritin in Gr 3 versus Gr 1 in men p value 0.02.

Use of IS for 6 months resulted in a small reduction in mean Hb in women in all the three groups and in two out of the three groups in men.

The fall in Hb in women in group 2 was significant. At 12 months of use of allocated salt in the RT the difference in Hb between group 1 and group 2 women was significant. Changes in mean ferritin levels following use of IS for six months were small and varied between men and women and between groups. There was an increase in ferritin in men in group 2 when they had used IS for 6 months which was statistically significant.

Changes in mean Hb, ferritin after 12 months of use of salt as per random allocation and use of purchased IS for 6 months during the lockdown by all the three groups in paired samples is given in Table 3. When paired samples between RT 12 months and LD 6 months were considered none of the differences in Hb and ferritin were significant. Inter-group differences in mean ferritin levels in women in group 2 and group 3 were higher at LD 6 months as compared to group 1; these differences in mean ferritin were statistically significant.

To assess whether baseline Hb had any impact on Hb response when DFS use was discontinued, women were classified into anaemic (<12 gm/dl) and non-anaemic (≥12 gm/dl) on the basis of Hb at 12 months of use of allocated salt. Comparison of Hb in paired samples at RT 12 months and LD 6 months is given in Table 4. The changes in mean Hb in both anaemic and non-anaemic women were small and statistically not significant.

Mean Hb and ferritin prior to enrolment in the randomized trial was compared with the mean Hb and ferritin after all the three groups used IS for six months is given in Table 5.

The mean Hb in women in all the three groups were higher after 6 months of use of IS as compared to the enrolment. This is because in all the three groups there was an increase in mean Hb after twelve months of use of the salts provided as per random allocation and the fall in Hb because of 6 months use of the iodised salt was small. The increase in Hb in women in group 1 was statistically significant.

Comparison of the mean ferritin in men and women in all the three groups at enrolment and after use of IS by all the three groups for six months showed that both in men and women in all the three groups, there was a small increase in ferritin. The increase in ferritin in women in group 3 between enrolment and LD was statistically significant. The inter-group differences between group 3 and group 1 in ferritin after six months of use of IS in men was statistically significant (Table 5).

It is reassuring to note that when families who had used DFS for 12 months use IS for six months, the reduction in Hb or ferritin if any was small and not significant.

## DISCUSSION

The prevalence of iron deficiency and anaemia in India is high across all age, sex and physiological groups. This is mainly due to inadequate iron intake and poor bio-availability of iron from habitual Indian diets.[3] Food fortification is a feasible and sustainable intervention which can bring about improvement in the iron intake of the entire family without any alteration in food habits.[13,14] India has been a pioneer in the development and evaluation of iron fortified iodised salt.[5] Research studies in India and elsewhere have shown that use of either of the DFS formulations resulted in some improvement in Hb in children.[7-10]

Data from our community based randomized study on impact of DFS use on Hb and ferritin showed that DFS use for 12 months resulted in an improvement in mean Hb in children and women.[12] Improvement in Hb was higher in anaemic women and children. There was no change in mean Hb in non-anaemic persons. There was a small improvement in ferritin in women who have low ferritin but no change in ferritin in women and men with normal ferritin.[12] The study was terminated after 12 months of use of the allocated salt because of the COVID related lockdown between April and September 2020.

During lockdown majority of the migrant families left for their villages. When the research team went back to the community in October 2020, they found that the majority of those remaining in the area were families who were born and brought up in Delhi, had a stable small-scale business/service shop and/or owned their houses in Delhi. The educational and occupational profile of the families remaining in the area did not differ from the profile of the families who had left the area. Families remaining in the area had faced economic constraints. They reduced all non-essential expenditures. They accessed PDS for subsidized and free food grains during lockdown; at times they also accessed free hot cooked meals provided during the lockdown. There was a steep fall in their vegetable intake because vegetables were not readily available and were expensive. The fall in vegetable intake might have contributed to the fall in Hb seen in both women and men and in all the three groups. The reduction in iron intake because of use of IS might have been the major factor responsible for the fall in Hb in group 2 and 3 who had earlier used DFS for 12 months.

After twelve months use of DFS the mean Hb in women using DFS was higher as compared to the IS users. The magnitude of fall in Hb with six months of use of IS was lower in DFS users (group 2 and 3) as compared to IS users (Table 3). Data from randomized study on use of DFS for 12 months suggest that the impact of DFS on Hb is higher in women and children and in anaemic persons. Contrary to expectations, the fall in Hb with 6 months use of IS in all three groups was more in non-anaemic women as compared to anaemic women. These differences were not statistically significant, because of the small number

of non-anaemic women (Table 4). The data so far presented suggest that even when there are disruptions in supply of DFS for a period upto six months, the population who had used DFS continue to have higher Hb as compared to the population who had used IS. Studies need be taken up to find out what happens to Hb and ferritin levels in erstwhile DFS users when they discontinue the DFS use for 12 months or longer.

Currently there are no publications reporting the impact of DFS use for two years or longer on Hb and ferritin levels. It is important to find out whether the pace and magnitude of rise in Hb in the first 12 months is sustained when DFS is used for 2 years or longer. A beginning can be made by assessing the impact of DFS use for a further period of 12 months in erstwhile DFS group families.

***Strengths of the study***

The study utilized the six-month gap in the supply of DFS to families during lockdown in 2020, to investigate the impact of discontinuation of DFS use for six months on Hb levels in a cohort of families who had completed use of the allocated salt for 12 months.

Limitations of the study are many families who were part of the randomized study on impact of DFS use on Hb status and had completed using the allocated salt for 12 months had gone back to villages or moved out to other areas in Delhi. Majority of children stayed back in villages because schools were closed. As a result, the number of families and persons in whom the impact of use of IS for 6 months could be studied were relatively small.

## CONCLUSION

Research studies showed that use of iron fortified iodised salt (DFS) resulted in improvement in Hb in women and children. In a vast country like India there are difficulties in ensuring continued supply of DFS especially in the initial phases of the programme. There had so far not been any publications documenting the impact of stopping the use of DFS on Hb levels.

Our institution was conducting a community based, three-armed open randomised study of two formulations of DFS and iodised salt (control arm) to assess the impact of use of DFS on Hb and ferritin. During lockdown (April-September 2020) the research team could not supply salt to the families; families were requested to purchase iodised salt (IS) from the market and use it. The disruption in supplying DFS for 6 months provided an opportunity to assess the impact of stopping the DFS use on Hb levels.

Comparison of mean Hb after 12 months use of the allocated salt with Hb after six-month use of IS by all three groups showed that: (1) there was a small reduction in Hb in all three groups after using IS for 6 months; (2) women who had earlier used DFS had higher mean Hb as compared to those who had used IS, both after 12 months of use of DFS and after 6 months of use of IS; (3) changes in ferritin varied and were not consistent.

The impact of the higher mean Hb when they restart using DFS for further 12 months has to be investigated.

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