



Effect of iron-fortified wheat flour on hemoglobin levels among women of reproductive age group in Mansehra, KPK, Pakistan

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ABSTRACT

Micronutrient deficiencies continue to affect approximately 25% of the World's population. Fortification of staple foods is recognized as one of the most effective interventions to combat micronutrient deficiencies such as iron deficiency. The objective of the current research was to elucidate the effect of iron-fortified wheat flour on the mean hemoglobin levels of women of the reproductive age group (15-49 years) in the Mansehra district, KPK, Pakistan. The study sample consisted of 280 women whose baseline hemoglobin levels were determined at the start of the study. They were fed with iron-fortified wheat flour for a period of 120 days after which their hemoglobin levels were measured again. A 24-hour dietary recall was also taken from the study participants to determine the amounts and frequencies of major foods consumed during the last 24 hours. The study results showed that the consumption of iron-fortified wheat flour had significantly increased the mean hemoglobin levels of women. The study concluded that the consumption of iron-fortified wheat flour could be an effective strategy to combat the problem of iron deficiency in Pakistan.

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Introduction

Anemia exists in various forms such as Pernicious anemia, Megaloblastic anemia, Sideroblastic anemia and others. However, the most commonly occurring form of anemia is that of Iron Deficiency Anemia (IDA) which occurs because of low levels of iron which is needed to generate red blood cells (RBCs)(1). Globally, iron deficiency is the biggest micronutrient malnutrition and is a massive public health issue not only in underdeveloped and developing countries but also in developed countries and affects as much as 25% to 30% of the World's total population (2).

Both iron deficiency and iron deficiency anemia are extremely prevalent across populations, especially among women (3). The major cause of chronic anemia worldwide is known to be iron deficiency. In fact, it is believed that the most common global nutritional disorder is that of iron deficiency and contributes to 50% of anemic cases (4). As per the estimates of the World Health Organization, the prevalence of iron deficiency in industrialized countries is 15% (5).

With regards to the global prevalence of anemia, WHO estimates that 47% under 5 children while 30% of non-pregnant women of reproductive age are anemic. For pregnant women, the worldwide prevalence of anemia has been estimated to be 42% (6). According to the most recent find-

ings of Pakistan's National Nutrition Survey 2018, about 41.7% of Pakistani women of reproductive age are anemic. Similarly, according to the figures of the same survey, approximately 18.2% of Pakistani women are iron deficient (7).

Four major strategies have been found to be extremely effective against iron deficiency anemia and these include dietary diversification, iron supplementation, biofortification and food fortification (8). However, in areas where infectious diseases such as malaria remain to be endemic, the safety and efficacy of routine iron supplementation is still a question mark. Implementation of interventions focused on the control and prevention of iron deficiency anemia, especially in developing countries calls for cautious epidemiological assessment, selection of appropriate techniques which would go well with the particular region and the residing population, and continuous monitoring so as to make certain the effectiveness as well as the safety (9).

Iron fortification is not only beneficial in reducing the problem of iron deficiency but it can also hinder the absorption of heavy metals such as Lead. Bouhouch *et al.* conducted a study in which the objective was to evaluate the effects of NaFeEDTA and FeSO₄ on iron status as well as the reduction of blood lead levels. The results of the study showed that when iron-fortified and lead-exposed children were given biscuits fortified with NaFeEDTA and

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FeSO₄, their iron status was significantly improved (P-value < 0.05). Moreover, it was also found that blood lead concentrations of such children were also significantly reduced (P-values < 0.05) (10).

Several food products are in use as vehicles for the purpose iron fortification, however, wheat flour has been the most common of these (11). As per most updated records, around 80 countries across the globe fortify flour yet the prevalence of iron deficiency anemia even in these countries has not substantially decreased (12).

Wheat is recognized to be the staple food of the Pakistani community and provides around 50% of their overall energy intake (13). Back in 2007, the wheat flour fortification program was launched in the Khyber Pakhtunkhwa province of Pakistan, but now it has been spread countrywide. In total, Pakistan has initiated 7 wheat flour fortification campaigns during the last 15 years, two of which have been on the national level (14).

Efforts are now being made by the private sector in collaboration with the government of Pakistan to spread awareness among people regarding the benefits of consuming iron-fortified wheat flour. This would hopefully lead to the improved nutritional status of the Pakistani population (11).

The major objective of the study was to elucidate the effect of iron-fortified wheat flour consumption on hemoglobin levels of women of the reproductive age group (15-49 years) in the Mansehra district of Khyber Pakhtunkhwa province, Pakistan.

Materials and Methods

Study settings

The study was conducted in the Mansehra district of Khyber Pakhtunkhwa, Pakistan which has four Union Councils. Information about the residents of the area was obtained from each of the Union Councils. For the purpose of obtaining a representative sample, 70 study participants were selected from each Union Council through the process of simple random sampling.

Study population

The study population consisted of women of the reproductive age group (15-49 years) from each of the four Union Councils of Mansehra district, Khyber Pakhtunkhwa, Pakistan.

Inclusion criteria

All women of the reproductive age group who were willing to take part in the study and give their blood samples were included in the study.

Exclusion criteria

Women who were pregnant or lactating, those above the age of 49 years or having some chronic medical conditions were excluded from the study.

Sample size

All the families were considered for the study as having women of reproductive age. Families having young girls, pregnant/lactating and elderly women were excluded from the sample size. Total of 287 women were initially selected for the purpose of our study. However, n = 7 women

refused to take part in the study as they did not want to give their blood samples. Hence our sample size eventually comprised of 280 women.

Data collection

The study continued for a period of 120 days (that is, four months). Baseline hemoglobin levels of the women who participated in the study were taken at the initiation of the study. After that, the hemoglobin levels of women were checked on the 120th day, at the termination of the study.

Iron deficiency anemia was diagnosed on the basis of the Hemoglobin levels of study participants as Hemoglobin is the most commonly recommended test for the detection of Iron Deficiency Anemia worldwide. Since it was not feasible to take blood samples of selected women from the community and bring it to the laboratory for further analysis, hemoglobin levels were determined using HemoCue B-Hemoglobin Fotometer (HemoCueAB, Angelholm, Sweden), as per the respective protocol (15).

For the purpose of determining of average consumption of various food items, a 24-hour dietary recall was taken from the study participants.

Calculations for iron in fortified wheat flour

It was important to know the actual consumption of fortified wheat flour and the availability of iron to the selected women; therefore, Electronic Food Measuring Scale was used to weigh chapatti/paratha in grams. The portion size was measured using standardized utensils, in order to estimate the quantity of other food items consumed.

17 mg additional iron per pound was present in the wheat flour after fortification (Nicholas Piramal India Limited – Certificate of Micronutrient Premix Analysis, 2006). When converted to kilograms, the value became 37.7mg of iron per 1000g or 3.77mg per 100g wheat flour. 4.5mg of iron is already present in per 100g wheat flour (16). By adding these two values the total iron present in the fortified wheat flour came out to be as 8.27mg/100g.

100g fortified wheat gives iron = 8.27mg
 1g fortified wheat gives iron = $8.27 \div 100 = 0.0827$
 $*343.5\text{g fortified wheat gives iron} = 0.0827 \times 343.5 = 28.4\text{mg}$ (*Average intake of wheat by research participants)

The recommended dietary allowance (RDA) of iron for Pakistani women of child-bearing age is 30mg/day (16).

Data analysis

SPSS version 23.0 was used for the purpose of data entry and analysis. After data cleaning, frequencies and percentages of variables were calculated using descriptive statistics. Paired t-test was applied to determine if the consumption of iron-fortified wheat flour had significantly raised the hemoglobin levels of women or not. Data were considered to be significant at a P-value less than 0.05 (17).

Ethical considerations

Informed consent of women was obtained prior to data/blood collection. The names and identities of all the women were kept confidential throughout the study and data was not revealed to anyone. Moreover, prior ethical approval of the study was taken from SAARC Human Resource Development Center vide letter no. SHRDC/ERB/2015/24.

Table 1. Mean consumption of various food items by women.

S. No.	Food Item	Consumption per day (g)	Calories	Protein (g)	Iron (mg)
1	Wheat (Roti)	343.5	890	30.2	28.4
2	Maize	9.3	26	0.9	0.2
3	Rice	115.7	189	3.7	0.7
4	Pulses/Legumes	24.9	43	3.0	0.4
5	Fat	31.96	286	0.0	0.0
6	Meat	27.7	55	5.15	0.6
7	Egg	4	7	0.5	0.1
8	Milk (mL)	13.8	12	0.5	0.03
9	Tubers	31.8	17	0.5	0.2
10	Dark green leafy vegetables	18.3	8	0.5	1.2
11	Other Vegetables	7.6	6	0.7	0.3
12	Tea (mL)	410.4	70	3.2	0.2
13	Sugar	45.2	176	0.0	0.3
Total			1785	48.85	32.63
N = 280					

Results

Mean consumption of food items by 24-hour dietary recall

Results related to mean consumption of various food items by women of childbearing age are given in Table 1.

Estimation of hemoglobin levels of women

At the start of the study, the mean hemoglobin levels of the study participants were determined to be as 10.20 g/dL. Severe anemia (Hb <7g/dL) was present among 16.7 percent of surveyed women while moderate anemia (Hb 7.0-11.9g/dL) was found in 63.0 percent of women. 20.0 percent of total women were found to have normal hemoglobin levels (Hb >12g/dL) and were free of anemia (table 2).

Hemoglobin levels of women were also determined on the 120th day of the study. The mean hemoglobin levels for the study participants came out to be as 11.85g/dL. Severe anemia was absent among the women surveyed. Moderate anemia was present among 67.5 percent of the women. 32.5 percent of the women had normal hemoglobin levels (table 3).

Discussion

Mean consumption of food Items by 24-hour dietary recall

In our study, consumption of wheat flour in form of non-leavened roti or paratha was found to be 343.5g / woman/day, providing 890 calories, 30.2 g protein and 28.4 mg of iron. Maize consumption was not much, on average it was 9.3g/day, providing 26 calories, 0.9g of protein and 0.2mg of iron.

Rice was the second largest portion of staple food consumed by the women, after wheat. It was eaten once a day by most of families. Daily intake of rice was 115.7g /woman which provided 189 calories, 3.7g protein and 0.7g iron. The pulses and legumes consumed by the women were not much. On average it was 24.9g, which gave 43 calories, 3g of protein and 0.4g of iron.

Fats and edible oils were the major sources of energy

Table 2. Hemoglobin Levels of Women of Reproductive Age Group at 0 Day.

Hemoglobin Status	Hemoglobin (g/dL)	Percent Women
Severe Anemia	< 7	16.7
Moderate Anemia	7 – 8.99	6.7
“	9 – 10.99	19.2
“	11 – 11.99	37.1
Normal	12 – 13.99	15
“	14 – 15.99	5
N = 280		

Table 3. Hemoglobin Levels of Women of Reproductive Age Group at 120th Day.

Hemoglobin Status	Hemoglobin (g/dL)	Percent Women
Severe Anemia	< 7	0.0
Moderate Anemia	7 – 8.99	1.4
“	9 – 10.99	28.2
“	11 – 11.99	37.8
Normal	12 – 13.99	26.0
“	14 – 15.99	6.4
N = 280		

for these women. Fat was contributing 286 calories to the diet of the research participants. The consumption of animal protein (beef, mutton and chicken) was considerably low 27.7g as compared to the national intake of 72g / person/day, providing 55 calories, 5.15g of protein and iron 0.6mg. Like meat, the consumption of egg was also very rare. On average, egg intake was 4g/person/day, contributing 7 calories, 0.5g protein and 0.1mg iron.

On average milk, consumption was 13.8ml / woman/day, which was significantly low when compared to the national figures of 90ml / person/day (NNS 2001-02). This small quantity of milk provided only 12 calories, 0.5g of protein and a negligible amount of iron. The tubers consumed by this set of population were potatoes and turnips. Their average intake/person/day was 31.8g, providing 17

calories, 0.5g of protein and 0.2mg of iron.

Consumption of leafy vegetables (spinach and saag) turned out to be 18.3 g / day which provided 8 calories, 0.5g protein and 1.2 mg iron. This is an iron-rich food group, but the consumption was very poor as compared to consumption of 57 g /person/day as mentioned in the NNS 2001-02.

Vegetables other than DGLV were eaten 7.6g /day. This quantity provided 6 calories, 0.7g protein and 0.3mg iron. None of the respondents had eaten fruits in the last 24 hours.

Intake of tea was high due to winters and on average 410.4ml/day. No significant nutrients are present in tea; the calories and protein provided by tea were due to added milk and sugar. Intake of sugar was 45.2g / woman/day, providing 176 calories and 0.3mg of iron.

Comparison of hemoglobin levels of women of reproductive age at 0 and 120th Day

According to the results presented in figure 1, severe anemia was present in 16.7% women at the start of study which decreased to 0 percent at the termination of study. Moderate anemia was present among 63% women at the start. However, when hemoglobin levels were determined at the termination of study, 67.5% women were found to be suffering from moderate anemia. The reason for this increase in percentage of women suffering from moderate anemia was that women who had severe anemia at the start of study had improved hemoglobin levels due to consumption of iron-fortified wheat flour. This means that a significant percentage of women with severe anemia at the start of study were categorized as having moderate anemia at the end of study, which indicated improvement in hemoglobin levels of women. Similarly, 20% women had normal hemoglobin levels at the initiation of study which raised to 32.5% at the end of study (figure 1).

When paired t-test was applied to compare the prevalence of severe anemia among women at 0 and 120thday, it was found that there was a significant difference in the scores (-16.70 ± 2.56); $t = -38.44$ (P-value < 0.05). Also for moderate anemia, again a statistically significant difference in the scores (-3.23 ± 1.19); $t = -4.57$ (P-value < 0.05) was found.

As is evident from the 24-hour dietary recall of women, they consumed fortified wheat as the larger portion of their staple food so the iron intake was also higher during the 120-day study period. This resulted in improved hemoglobin levels from 0 days at the start of the study to 120th days at the termination of the study.

The results of a systematic review and meta-analysis of effects of iron-fortified wheat flour on various iron biomarkers for pre and post-studies showed that there was a significant increase in mean hemoglobin levels (3.360 g/L; 95% CI: 0.980, 5.730) while a significant decrease in iron deficiency (-10.4%; 95% CI: -14.3, -6.5) (18). This closely resembles the results of our research study whereby we have also reported increased mean hemoglobin levels among women of reproductive age after they consumed iron-fortified wheat flour for a period of 120 days.

According to the evidence generated from a systematic review on the effectiveness of wheat flour fortification programs on iron status, it was reported that there was a significant decrease in anemia among 4 out of 12 sub-groups of women of reproductive age when they consumed iron-

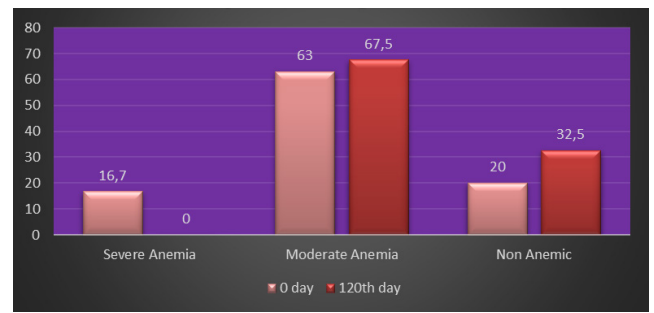


Figure 1. Comparison of hemoglobin levels (g/dL) of women of reproductive age at 0 and 120th day.

fortified wheat flour on regular basis (19).

As per the results of a study conducted in Cameroon, it was observed that when women were fed with iron-fortified wheat flour, there was a significant decrease in maternal anemia from 46.7% to 39.1% (P-value < 0.01) (20), which is in close resemblance with our study results. Also, the food fortification program of Costa Rica resulted in significantly decreased anemia levels among women from 18.4% (95% CI; 15.8, 20.9) to 10.2% (95% CI; 8.2, 12.2) (21).

It is evident from the findings of the present study that iron-fortified wheat flour was able to improve mean hemoglobin levels among women of the reproductive age group. The main reason was that iron-fortified wheat flour provided ample amounts of iron which was quite near to the RDA despite. Thus, it can be concluded that wheat flour fortification is a cost-effective intervention to combat the menace of iron deficiency among women of reproductive age group specifically in a developing country such as Pakistan.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Riffat Aysha Anis and Abdul Momin Rizwan Ahmad conceptualized the study. Mariam Anees and Riffat Aysha Anis collected the data from the field. Saira Zafar, Umar Farooq and Juweria Abid performed data analysis while Riffat Aysha Anis and Sajeela Akram wrote the initial manuscript. Umar Farooq and Mariam Anees did the final write-up.

References

- Ahmad AMR, Ahmed W, Iqbal S, Mushtaq MH, Anis RA. Synergistic effect of galacto oligosaccharides and iron fortificants on serum iron, ferritin, transferrin and total iron binding capacity levels in anemic rats. *Pak J Pharm Sci.* 2019;32(5 (Supplementary)):2205-13.
- dos Santos Vieira DA, Steluti J, Verly-Jr E, Marchioni DM, Fisberg RM. Brazilians' experiences with iron fortification: evidence of effectiveness for reducing inadequate iron intakes with fortified flour policy. *Pub Health Nutr.* 2017;20(2):363-70.
- Frater JL. The Top 100 Cited Papers in the Field of Iron Deficiency in Humans: A Bibliometric Study. *BioMed Res Int.* 2021;2021.
- Pasricha S-R, Tye-Din J, Muckenthaler MU, Swinkels DW. Iron deficiency. *The Lancet.* 2020.
- Garzon S, Cacciato PM, Certelli C, Salvaggio C, Magliarditi M, Rizzo G. Iron deficiency anemia in pregnancy: novel approaches

- for an old problem. *Oman Med J.* 2020;35(5):e166.
6. Sun J, Wu H, Zhao M, Magnussen CG, Xi B. Prevalence and changes of anemia among young children and women in 47 low-and middle-income countries, 2000-2018. *E Clin Med.* 2021;41:101136.
 7. Ahmed J, Khan MT, Hameed B. Hematological profile in patients with chronic kidney disease in Pakistan: a cross-sectional research study. *J Egyptian Soc Nephrol Transplant.* 2021;21(1):57.
 8. Ahmad AMR, Ahmed W, Iqbal S, Javed M, Rashid S, ul Haq I. Prebiotics and iron bioavailability? Unveiling the hidden association-A review. *Trends Food Sci Technol.* 2021.
 9. Benson C, Shah A, Stanworth S, Frise C, Spiby H, Lax S, et al. The effect of iron deficiency and anaemia on women's health. *Anaesthesia.* 2021;76:84-95.
 10. Bouhouch RR, El-Fadeli S, Andersson M, Aboussad A, Chabaa L, Zeder C, et al. Effects of wheat-flour biscuits fortified with iron and EDTA, alone and in combination, on blood lead concentration, iron status, and cognition in children: a double-blind randomized controlled trial. *The Am J Clin Nutr.* 2016;104(5):1318-26.
 11. Ahmad AMR, Ahmed W, Iqbal S, Mushtaq MH, Anis RA. Iron and prebiotic fortified flour improves the immune function of iron deficient women of childbearing age. *Pak J Pharm Sci.* 2020;33.
 12. Barkley JS, Wheeler KS, Pachón H. Anaemia prevalence may be reduced among countries that fortify flour. *Br J Nutr.* 2015;114(2):265-73.
 13. Ishaq A, Khalid M, Ahmad E. Food insecurity in Pakistan: A region-wise analysis of trends. *Pakistan Institute of Development Economics (PIDE) Working Papers.* 2018;157.
 14. Ansari N, Mehmood R, Gazdar H. Going against the grain of optimism: flour fortification in Pakistan. 2018.
 15. Rappaport AI, Karakochuk CD, Hess SY, Whitehead Jr RD, Namaste SM, Dary O, et al. Variability in haemoglobin concentration by measurement tool and blood source: an analysis from seven countries. *J Clin Pathol.* 2021;74(10):657-63.
 16. UNICEF. Food Composition Table for Pakistan (revised 2001). Islamabad, Pakistan[Online]. 2001.
 17. Anderson VL, McLean RA. Design of experiments: a realistic approach: CRC Press; 2018.
 18. Sadighi J, Nedjat S, Rostami R. Systematic review and meta-analysis of the effect of iron-fortified flour on iron status of populations worldwide. *Pub Health Nutr.* 2019;22(18):3465-84.
 19. Pachon H, Spohrer R, Mei Z, Serdula MK. Evidence of the effectiveness of flour fortification programs on iron status and anemia: a systematic review. *Nutr Rev.* 2015;73(11):780-95.
 20. Engle-Stone R, Nankap M, Ndjebayi AO, Allen LH, Shahab-Ferdows S, Hampel D, et al. Iron, zinc, folate, and vitamin B-12 status increased among women and children in Yaounde and Douala, Cameroon, 1 year after introducing fortified wheat flour. *J Nutr.* 2017;147(7):1426-36.
 21. Martorell R, Ascencio M, Tacsan L, Alfaro T, Young MF, Addo OY, et al. Effectiveness evaluation of the food fortification program of Costa Rica: impact on anemia prevalence and hemoglobin concentrations in women and children. *Am J Clin Nutr.* 2015;101(1):210-7.