

**A STUDY ON CORRELATION PHYTATE LEVEL IN
COOKED RICE AND AVERAGE IRON IN DIET
WITH BLOOD INDICES IN URBAN SETTLEMENTS
OF CHENNAI**

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ABSTRACT

INTRODUCTION: Iron deficiency anaemia is more prevalent in India. Nutritional deficiency is one of the common causes. Rice is the staple food item in the south Indian diet. Iron available as heme and non-heme forms in the diet. Iron absorption depend various physiological conditions and chemical nature of food items. Phytates are inositol hexakisphosphates. It is present in most of the cereals and legumes. Phytates form anions in intestinal pH which can bind divalent metal ions such as iron, calcium etc., Iron bioavailability is important in screening and diagnosis of nutritional anaemia. Based on this aim of our study is to assess the phytate level in cooked rice and iron bioavailability and association with blood Iron status.

Materials and Methods: 100 households were selected from ward 49 of zone 5 of Chennai Corporation through multistage random sampling technique. Male and female participants between 18 and 50 years of the households are selected based on inclusion and exclusion criteria. Duration of the study was 18 months. Blood samples collected for complete blood count, peripheral smear, serum ferritin and cooked rice samples collected for phytate analysis in research lab of department of Biochemistry. The average iron levels in diet and phytate levels in the cooked rice samples are correlated with the blood iron status of the participants.

Results: Demographically majority of the participants were belonged to middle class and practicing non-vegetarian diet. Symptoms and signs were seen only in few subjects. Most of them were moderately built and nourished. Mean phytate levels in cooked rice had no statistical significance on comparison with blood iron parameters. The mean average iron

levels in diet are not statistically significant comparing with blood iron parameters except haemoglobin, which had positive linear relationship.

Conclusion: The study concludes that in the selected population the mean phytate levels in cooked rice and the mean average iron levels in diet had no major impact on causing iron deficiency anaemia. Further detailed studies needed to ascertain the proposed theory.

Keywords: Anaemia, Phytate, cooked rice.

INTRODUCTION

Iron deficiency and iron deficiency anaemia are not synonyms. Iron deficiency is defined as a haemoglobin concentration below the optimum value in an individual. Iron deficiency anaemia is diagnosed when concentration of haemoglobin is below the 95th percentile of the distribution of concentration of haemoglobin in a population irrespective of effects of altitude, age, and sex, etc. on haemoglobin concentration. There is very wide distribution of the haemoglobin concentration in fully iron-replete healthy subjects (in men, 140–180 g/L in women; 120–160 g/L).¹

Iron bioavailability, defined as the extent to which iron is absorbed from the diet and used for normal body functions. There is no mechanism in human body for iron excretion and around 90% of daily iron needs are met from an endogenous source (red cells' destruction). Iron losses occur which include obligatory losses in all of population (intestines, urinary tract, skin and airways) and losses as menstrual blood in child-bearing age group of women. Iron balance should be maintained. For that in addition to the losses, Diet must provide the iron required for growing infants and children. Also adolescents, pregnant women should be supplied through diet.

A study estimated loss of iron in men with normal iron status from South Africa, the United States, Venezuela, an average of around 0.9–1.0 mg/day or 14 µg/kg body weight) from a reliable quantitative data for obligatory iron losses². Bothwell et al³ gave supportive evidence for this estimate of iron losses per kilogram of body weight based on daily losses. But Janet R Hunt et al studied that the obligatory iron losses vary with iron status and age, ethnicity and sex. So there is a need to evaluate further the extent⁴ The Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) and other organizations have estimated the iron requirements for different groups of population on the basis of iron needed for growth and the sum of menstrual and obligatory iron losses.¹

There are two types of dietary iron: non-heme iron and heme iron. Non-heme iron is present in both plant foods and animal tissues. Heme iron comes from haemoglobin and myoglobin in foods from animal source. In meat-eating populations, heme iron holds 10–15% of total iron intake. Because of its more uniform and higher absorption (estimated at 15–35%), it would contribute $\geq 40\%$ of total absorbed iron.⁵ Heme iron is usually well absorbed than non-heme iron. Depending on the balance between the inhibitors and enhancers of absorption and individual's iron status. Absorption is same for all non-heme food iron which enters the common iron pool in the digestive system.

In diets based on plants, phytate (myo-inositol hexakisphosphate) one of the main inhibitor of iron absorption. Dose dependent negative effect of phytate on iron absorption

has been documented. It starts at very low concentrations of 2–10 mg/meal.^{5,6} Al Hasan et al using multivariate models, studied that with phytate intake, inadequate micronutrient intake, and energy intake significantly predicted the variance in phytate to iron was 88 %.⁷

Cereals like rice (*Oryza sativa*) and finger millet (*Eleusine coracana*), pulses includes chickpea (*Cicer arietinum*), green gram (*Phaseolus aureus*) and black gram (*Phaseolus mungo*) form common food items in diet of Indian population.⁸ Chemically, phytates are inositol hexaphosphate salts. Phytate and other lower inositol phosphates (e.g., bran products, bread made from high extraction flour, breakfast cereals, oats, rice especially unpolished rice, pasta products, cocoa, nuts, soya beans, and peas) are a storage form of phosphates and minerals. These form part of the inhibiting factors of mineral absorption especially iron, calcium, etc., in turn affect the bioavailability. Phytates strongly inhibit iron absorption in a dose-dependent fashion and even small amounts of phytates have a marked effect.¹

Oliver Didzun et al found that in India, an estimated 21.7% of men with any degree of anaemia had moderate or severe anaemia compared with 53.2% of women with any anaemia in states of Bihar and Manipur⁹. The individual-level predictors of higher probability of anaemia were studied to be living in a rural area, lower education, less household wealth, consuming tobacco, smoking, and being underweight. The district-level predictors of living with a lower rate of primary school completion, household wealth, and level of urbanisation were all also associated with it.¹⁰ Based on this main aim of our study is to assess the phytic acid level in cooked rice, calculate average iron intake and its association with blood Iron status of Participants.

MATERIAL & METHODS

This study was done in Government Stanley Medical College involving department of Biochemistry and department of Pathology for a period of 18 months (May 2020 – October 2021) and was done as a community based Cross sectional study. Participants from both sexes in the 100 households of Zone 5 division 49 of Chennai Corporation with age between 18-50 years are included as cases of this study. No Paediatric participant's involved. Participants with age of less than 18 years and more than 50 years, anaemic and sick and with coexisting chronic diseases were excluded.

After obtaining informed consent, standardized proforma to study dietary iron intake used. Daily Dietary intake was noted. Frequency and quantity of intake of Heme and Non Heme Iron rich foods are also assessed. Iron content of the Food item is calculated using the National Institute of Nutrition (NIN) guidelines.

From these data average daily iron intake of the participant calculated. Cooked rice sample of the household being transported to research laboratory and analyzed for the amount of phytates using colorimetric method in Peripheral smear prepared using Leishman stain and study done using light microscopy. Serum ferritin measurement done using cobase e 411 analyzer.

The collected data were analysed with IBM.SPSS statistics software 23.0 Version. To describe about the data; Percentage analysis were used for categorical variables, Unpaired sample t-test was used to find the significant difference between the bivariate samples

among the independent groups. Pearson's Correlation was used to assess the relationship between the variables;

RESULTS

In our study of 230 patients majority of participants in the study (n=176, 76.5%) were in the age group 35-50 and rest were below that. In the study 115 male and 115 female participants were interviewed and collected biological specimens.

Out of the total participants, 27.5% (n=63) attended high school and 27% had middle school education. 16.5% (n=38) had higher secondary education whereas 15% went for primary school. Graduates were about 10% (n= 24) and 4% (n=9) were illiterate. According to BJ Prasad recent socioeconomic classification, participants divided into 5 classes. 42.5% (n=98) formed upper middle class and 38% (n=88) were middle class. Lower middle class were 15% (n=34) and upper class were 4.5% (n=10). None belonged to lower socioeconomic class. Among the whole participants, 30 had habit of taking alcohol and smoking habit seen in 19 participants. Only 3 had habit of chewing betel nut or tobacco.

Majority of the participants i.e. 86% (n=198) were moderately built. 10% (23) were well built and only 4% (n=9) were poorly built. Among the participants, 86.07 % (n=198) were moderately nourished, 4.8% (n=11) were poorly nourished and 9.13% (n=21) were well nourished. Out of total participants, 45 had fatigue, 17 had giddiness, 15 had headache. Only 3 had palpitation, chest pain and palpitation found in single participant. Pallor was found on examination in 35 participants. Jaundice, lymphadenopathy, pedal oedema were not evident in any participant. According to WHO classification, Majority of the participants i.e. 67% (n=154) had normal BMI. 16.5% of the participants were overweight, 11% were under weight and 13% were obese.

Coming to blood parameters normal RBC count was seen in 37% (n=85) who were males and 40% (n=92) who were females.13% (n=30) of the participants who were males and 10% (n=23) of them who were females had decreased RBC count. Also 18.5% (n=42) and 28.5% (n=66) of the participants who are male and female subjects respectively had decreased haemoglobin levels. The remaining participants i.e. 31.5% (n=73) who were males and 21.5% (n=49) who were females had normal levels of haemoglobin. Among the total participants, 28.5% (n=65) and 29% (n=67) had decreased haematocrit who were male and female subjects respectively. Rest of them i.e. 21.5% (n=50) who were males and 21% (n=48) who were females had normal haematocrit.

In our study 33% (n=76) of the total participants had reduced MCV levels and remaining 67% (n=154) had normal levels of MCV. Out of the total participants, 21% (n=48) had reduced MCH levels and rest of them i.e. 79% (n=182) had normal MCH levels. Among the total participants, 5.5% (n=13) had reduced levels of MCHC. The remaining 94.5% (n=217) had normal MCHC levels. We also analysed serum ferritin and among our study participants, only 5% (n=11) had decreased ferritin levels. All of them were females. None of the male participants had decreased ferritin levels.

In our study majority of the participants i.e. 41% (n=95) showed normocytic normochromic picture and 35% had normal study. Microcytic hypochromic picture seen in 12% (n= 28) and macrocytic hypochromic picture found in 1% of the total participants. 11%

(n=25) of the participants had dimorphic picture and remaining 35% (n=80) of them came under normal study.

Table 1: Peripheral smear levels among study population n=230

PERIPHERAL SMEAR	NO OF PARTICIPANTS	PERCENTAGE
NORMAL STUDY	80	35.00%
NORMOCYTIC NORMOCHROMIC	95	41.00%
MICROCYTIC HYPOCHROMIC	28	12.00%
MACROCYTIC HYPOCHROMIC	2	1.00%
DIMORPHIC	25	11.00%

We further analysed the correlation between phytate levels and average iron content in diet with the different RBC parameters. Correlation of mean phytate levels in cooked rice and blood iron indicators by Pearson’s Correlation shows no statistical significant positive linear relationship between mean phytate level and RBC count, RDW_CV, and serum ferritin levels Whereas Correlation between mean phytate levels in cooked rice and blood iron indicators by Pearson’s Correlation shows no statistical significant negative linear relationship between mean phytate levels in cooked rice and haemoglobin, haematocrit, MCV, MCH, and MCHC.

Table 2: Mean phytate level and average iron in diet in comparison with blood indices

PEARSON CORRELATION		PHYTATE LEVELS IN DIET	AVERAGE IRON IN DIET
RBC	Pearson Correlation	0.029	0.024
	Sig. (2-tailed)	0.658	0.716
	N	230	230
HB	Pearson Correlation	-0.067	.215**
	Sig. (2-tailed)	0.309	0.001
	N	230	230
HCT	Pearson Correlation	-0.096	0.102
	Sig. (2-tailed)	0.147	0.124
	N	230	230
MCV	Pearson Correlation	-0.035	-0.017
	Sig. (2-tailed)	0.594	0.797
	N	230	230
MCH	Pearson Correlation	-0.025	0.122

	Sig. (2-tailed)	0.706	0.065
	N	230	230
MCHC	Pearson Correlation	-0.025	0.091
	Sig. (2-tailed)	0.701	0.167
	N	230	230
RDW_CV	Pearson Correlation	0.021	-0.063
	Sig. (2-tailed)	0.750	0.338
	N	230	230
S_Ferritin	Pearson Correlation	0.075	-0.049
	Sig. (2-tailed)	0.260	0.458
	N	230	230

DISCUSSION

In our study we aimed to find the relationship between the effects of phytate available in cooked rice, which is the major food item of south Indian diet, on the iron bioavailability of normal adults. Most of the adult population in India were not screened for iron deficiency anaemia. Iron deficiency anaemia is common among Indian population due to various demographic and socioeconomic factors. So iron deficiency anaemia goes unnoticed in adults unless clinical symptoms and signs sets in. Moreover, Nutritional iron deficiency is the commonest cause of iron deficiency anaemia in developing countries.

The study enrolled 230 adults which comprised 115 male subjects and 115 female subjects after careful verification of inclusion and exclusion criteria. Among male subjects, most of them belonged to Hindu religion (90%) in which mixed diet pattern is common may be related to the finding of 83.5% of the subjects was practicing non-vegetarian diet including meat, fish, egg. Hence in majority of the participants, religious beliefs are not hindering the intake heme iron containing food.

Regarding education, most of them were literate who acquired school education such as primary (15%), middle (27%), high (27.5%), higher secondary (16.5%) indicating the food taboos may be eliminated to some extent with additional factor of urban dwelling feature.

Most of them (76.5%) were above 35 years of age that implies they were potential earning members (57.5%) except the remaining participants (42.5%) in which housewives, unemployed were included. Hence preparation of various kinds of food items and consumption among the subjects are well practiced.

Regarding nutritional assessment, most of them were moderately built (86%) and nourished (86.07%). BMI among the total participants classified as normal (67%) among majority of the participants. Remaining 16.5% were overweight, 11% were underweight and 5.5% were obese.

On elucidating the present medical history of the participants, 45 of them had fatigue, 17 had giddiness and headache featured among 15 participants. Palpitation, chest pain, loss

of appetite was documented rarely. On clinical examination, 35 of the participants had pallor and other general signs not present among the total subjects. Systemic examination found to be normal in all subjects. Consolidating the findings, symptoms and signs of anaemia such as fatigue, giddiness, headache, pallor in considerable number of subjects. Hence the underlying prelatent or latent iron deficiency is unnoticed among common adult population. This will indirectly affect their productivity and quality of life in turn have the impact on their family wellbeing.

Among the male subjects (n=115), majority of them had normal RBC Count (n=85), Hb (n=73) but haematocrit was found to be less than normal among 65 participants may be due to occupational influence. Serum ferritin was normal among all male subjects' shows that anaemia is not evident among major male participants. Among female subjects (n=115), most of them (n=92) had normal RBC Count but decreased Haemoglobin (n=66) and haematocrit (n=67) that implies latent iron deficiency is prevalent. Serum ferritin found to be low in 4 female subjects which adds evidence to the finding.

Regarding indices like MCV, MCH, MCHC, RDW_CV were resulted as normal among 67%, 79%, 94.5%, and 73.5% of the participants respectively. But peripheral smear showed normochromic normocytic (41%) and microcytic hypochromic (12%) picture among the total subjects. Both together attribute to the finding that iron deficiency can be attributed among more than 50% of them which is unnoticed usually by general population.

The mean phytate levels measured from cooked rice samples were compared with participants' blood iron indicators taken for the study such as RBC Count, Hb, Hct, MCV, MCH, MCHC, RDW_CV, serum ferritin, and peripheral smear using unpaired t-test and one way ANOVA wherever applicable, found to be MCH and peripheral smear study had significance, whereas rest of them were not significant. This implies that mean phytate levels in the rice samples had significant effect on haemoglobin content of the RBCs which indirectly shows that iron content availability for haemoglobin production in RBCs can be influenced by dietary phytates.

The mean average iron levels in diet calculated from food frequency questionnaire WHICH were compared with participants' blood iron indicators taken for the study such as RBC Count, Hb, Hct, MCV, MCH, MCHC, RDW_CV, serum ferritin, and peripheral smear using unpaired t-test and one way ANOVA wherever applicable, found to be RBC Count, haematocrit, ferritin and peripheral smear study had significance, whereas rest of them were not significant. This implies that mean average iron levels in diet had significant effect on erythropoiesis and storage form of iron in the participants which indirectly shows that dietary iron availability is important for production of RBCs and reserve stores in the body.

Correlation of mean phytate levels in cooked rice and blood iron indicators by Pearson's Correlation shows no statistical significant positive linear relationship between mean phytate level and RBC count, RDW_CV, and serum ferritin levels Whereas Correlation between mean phytate levels in cooked rice and blood iron indicators by Pearson's Correlation shows no statistical significant negative linear relationship between mean phytate levels in cooked rice and haemoglobin, haematocrit, MCV, MCH, and MCHC. This correlation collectively shows that in this study population, the mean phytate levels in

the cooked rice have no significant influence on blood iron parameters. This may be reasoned to the current trend of cooking polished rice with advent of science.

Correlation of mean average iron levels in diet and blood iron indicators by Pearson's Correlation shows no statistical significant positive linear relationship between mean average iron levels in diet and RBC count, haematocrit, MCH, MCHC but has significant correlation with haemoglobin measurement Whereas Correlation between mean average iron levels in diet and blood iron indicators by Pearson's Correlation shows no statistical significant negative linear relationship between mean average iron levels in diet and MCV, RDW_CV, and serum ferritin. This correlation collectively shows that in this study population, the mean average iron levels have no significant influence on blood iron parameters except haemoglobin quantification.

CONCLUSION

The mean phytate levels in cooked rice samples can influence the blood iron parameters but not of much significance in this study population whereas mean average iron levels in diet of the study population can influence the blood iron parameters but not of much significance except haemoglobin parameter. This finding proves the already known fact that despite presence of heme reserves, dietary iron which includes both heme iron and non-heme is essential for haemoglobin production in our body. Phytate is common constituent of cereals and legumes worldwide. Hence studying the effect of phytates on various mineral bioavailabilities can greatly improve the knowledge on quality food practices to eliminate mineral deficiencies from diet.

LIMITATION OF THIS STUDY

It's a community based study which involved only less number of participants. Most of our participants were above 30 years; hence age wise segregation and comparison would be better. Only phytates in cooked rice used to correlate the average dietary iron intake. The other metal ions which have similar properties on interaction with phytates are not taken into account.

REFERENCES:

1. Basta SS, Soekirman DS, Karyadi D, Scrimshaw NS. Iron deficiency anemia and the productivity of adult males in Indonesia¹ [Internet]. Vol. 32, The American Journal of Clinical Nutrition. 1979. Available from: <https://academic.oup.com/ajcn/article-abstract/32/4/916/4666354>
2. Al-Alimi AA, Bashanfer S, Morish MA. Prevalence of Iron Deficiency Anemia among University Students in Hodeida Province, Yemen. *Anemia*. 2018 Apr 23;2018.
3. Bothwell, T.H., and R.W. Charlton. 1981. *Iron Deficiency in Women*. Washington, D.C.: International Nutrition Anemia Consultative Group.
4. Hunt JR. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. *Am J Clin Nutr*. 2003 Sep;78(3 Suppl):633S-639S. doi: 10.1093/ajcn/78.3.633S. PMID: 12936958.
5. Zhang A-S, Enns CA. Molecular mechanisms of normal iron homeostasis [Internet]. *Hematology*. 2009. Available from: http://ashpublications.org/hematology/article-pdf/2009/1/207/645386/207_214ash.pdf

6. Conrad ME, Umbreit JN. A Concise Review: Iron Absorption-The Mucin-Mobilferrin-Integrin Pathway. A Competitive Pathway for Metal Absorption. Vol. 42, American Journal of Hematology.
7. Al Hassan NN. The prevalence of iron deficiency anemia in Saudi University female students. *J Microsc Ultrastruct.* 2015;3:25–28.
8. Loria A, Sanchez-medal L, Lisker R, Kodr E de, Labardini J. Red Cell Life Span in Iron Deficiency Anaemia. Vol. 294. 1967.
9. Didzun O, De Neve JW, Awasthi A, Dubey M, Theilmann M, Bärnighausen T, Vollmer S, Geldsetzer P. Anaemia among men in India: a nationally representative cross-sectional study. *Lancet Glob Health.* 2019 Dec;7(12):e1685-e1694.
10. Garrick MD, Garrick LM. Cellular iron transport. Vol. 1790, *Biochimica et Biophysica Acta - General Subjects.* 2009. p. 309–25.