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The Role Of HFE And TFR2 Gene Polymorphisms In Iron Homeostasis And Iron Deficiency Anaemia

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Abstract

'Iron deficiency anaemia (IDA)' is considered to be one of the most significant public health problems, not only in adolescent girls and pregnant women living in low- and middle-income countries. While nutritional factors remain the dominant cause, genetic variations in iron metabolism can modify individual susceptibility and influence treatment response. This study investigated the prevalence and clinical impact of HFE (C282Y, H63D) and *TFR2* gene polymorphisms in adolescent girls and expecting mothers from Rewa, Madhya Pradesh, India. A total of 520 participants (260 adolescent girls (between the ages of 10 and 18 years) and 260 expecting mothers (first or second trimester or less than 24 weeks during gestation) were assessed for 'hematological indices, serum ferritin, total iron-binding capacity (TIBC), and transferrin saturation (TS%)'. Genotyping was conducted using PCR–RFLP. The HFE C282Y variant was rare, but H63D heterozygosity was frequent and significantly associated with reduced ferritin and TS%, higher TIBC, and increased IDA risk. TFR2 variants showed limited direct association with IDA but may contribute through gene–gene interactions. These findings suggest that *HFE* H63D polymorphisms may exacerbate iron deficiency risk in high-demand physiological states, warranting genetic screening in anaemia control programs.

Keywords: HFE, TFR2, iron deficiency anaemia, gene polymorphism, iron homeostasis

1. Introduction

The most prevalent micronutrient deficiency condition, with 1.9 billion cases in the world, is IDA (World Health Organization [WHO], 2021). It poses a huge burden to diseases worldwide, especially in vulnerable populations like teenage girls, expectant mothers, and young children. IDA has adverse effects that include cognitive malformation, inhibited physical productivity, as well as an increase in maternal and perinatal deaths and poor immune response (Balarajan et al., 2011; Camaschella, 2015).Especially in India, the situation is very bad. The 'National Family Health Survey-5 (NFHS-5)' indicated that 57 percent and 59 percent of adult women and teenage girls aged between 15-19 and 15-49 years of age, respectively, are anaemic ('Ministry of Health and Family Welfare' [MoHFW], 2021). In Madhya Pradesh, prevalence rates are even higher, despite decades of iron supplementation and public health interventions. This suggests that nutritional deficiency alone may not fully explain the persistence of anaemia in these populations.

Human bodies have an iron homeostasis that is robustly regulated through a dearth of proteins that govern absorption, transport, utilisation, and storage. 'Hepcidin, a peptide hormone produced by the liver, is the system's master regulator of iron homeostasis and is known to modulate the function of ferroportin, the sole known cellular exporter of iron (Ganz, 2011)'.

'The HFE gene, located on chromosome 6p21.3', encodes a major histocompatibility complex 'class I-like protein that interacts with transferrin receptor 1 (TFR1)' to modulate hepcidin expression. The two most common polymorphisms, C282Y (845G>A) and H63D (187C>G), have been well studied in European populations due to their association with hereditary haemochromatosis (Beutler et al., 2002). While homozygosity for C282Y leads to iron overload, heterozygotes and H63D carriers may exhibit subtler changes in iron metabolism, which under conditions of increased iron demand (such as pregnancy or adolescence) could predispose to IDA.

The 'TFR2 gene, located on chromosome 7q22, encodes transferrin receptor 2', which is predominantly expressed in the liver and plays a key role in sensing circulating transferrin-bound iron (Girelli et al., 2016). TFR2 interacts with HFE and other iron-sensing proteins to regulate 'hepcidin transcription via the BMP/SMAD signalling pathway'. Pathogenic TFR2 variants can impair this iron-sensing mechanism, leading to dysregulated hepcidin production and altered iron availability (Gkouvatsos et al., 2012). Although severe loss-of-function mutations in TFR2 cause hereditary haemochromatosis type 3, more common single-nucleotide polymorphisms (SNPs) may exert milder effects that remain clinically relevant in certain physiological or nutritional contexts.

Emerging evidence indicates that genetic variations in HFE and TFR2 may influence the efficacy of iron supplementation and explain cases of refractory IDA, where anaemia persists despite adequate iron intake (Singh & Pandey, 2021). However, most research in this area has been conducted in European, East Asian, or North American populations, with limited data available for Indian cohorts. Given the distinct genetic architecture and dietary habits of the Indian population, studies exploring these variants in the context of IDA are crucial.

Therefore, the present study aimed to:

1. Determine the prevalence of HFE'(C282Y and H63D)' and selected TFR2 polymorphisms in adolescent girls and pregnant women from Rewa, Madhya Pradesh.

2. Assess their association with hematological parameters and biochemical indicators of iron status ('hemoglobin, serum ferritin, total iron-binding capacity, transferrin saturation').

3. Evaluate the risk of IDA associated with these genotypes, controlling for socio-demographic and dietary factors.

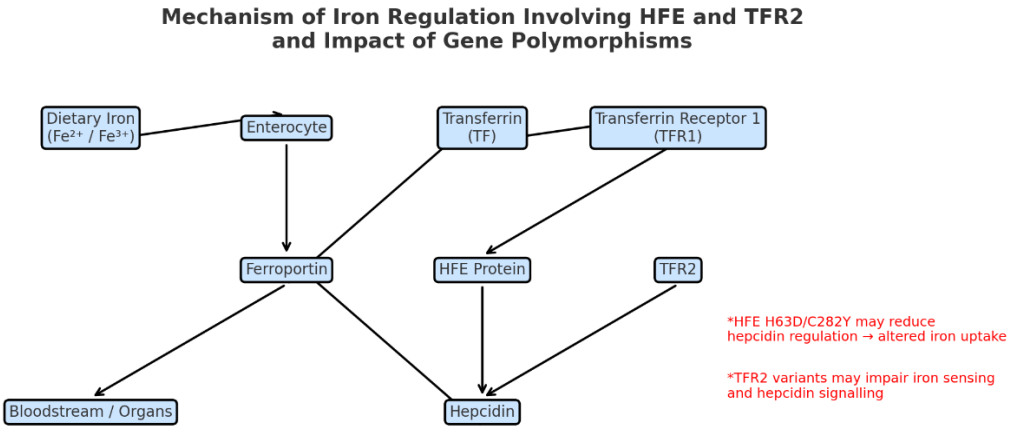
By integrating genetic screening into IDA research, this study seeks to provide insights into the multifactorial etiology of anaemia in high-burden regions and contribute to more targeted prevention and treatment strategies.

2. Literature Review

Dietary absorption, plasma transport in the blood, tissue storage, and recycling of the senescent erythrocytes are required to maintain iron homeostasis. Hepcidin is a hormone (produced in the liver) that regulates iron efflux through ferroportin found on the enterocytes and macrophages (Ganz, 2011). The impairment of this regulation may lead to an overload or a lack of iron. 'TheHFE gene,located on chromosome 6', encodes a transmembrane protein that modulates transferrin receptor 1 (TFR1) binding, thereby regulating hepcidin synthesis. The C282Y mutation disrupts protein folding, while H63D alters TFR1 interaction. Homozygous C282Y typically causes iron overload; however, heterozygous and compound heterozygous states have been associated with altered iron indices and susceptibility to IDA in high-demand conditions (Beutler et al., 2002; Merryweather-Clarke et al.,

2000). 'The TFR2 geneTFR2, located on chromosome 7', senses transferrin-bound iron and signals hepcidin transcription via the BMP/SMAD pathway. Mutations can impair hepcidin regulation, leading to either iron overload (type 3 haemochromatosis) or functional iron deficiency, particularly in inflammatory states (Girelli et al., 2016).

Although multiple studies in European and East Asian populations have explored these polymorphisms, Indian-specific data are limited (Teucher et al., 2004; Singh & Pandey, 2021).



3. Materials and Methods

3.1 Study Design and Setting

A case–control study design was implemented in Rewa, Madhya Pradesh, India, a region known for high anaemia prevalence among women of reproductive age. The study was conducted in collaboration with the Department of Biotechnology, '[University Name from your thesis], and received ethical approval from the Institutional Ethics Committee (Approval No. [from thesis data])'. All participants provided informed consent (or parental/guardian consent for minors).

3.2 Participant Recruitment and Inclusion Criteria

A total of 520 participants were enrolled, comprising:

Cases (n = 260): Adolescent girls (between the ages of 10 and 18 years) and Controls (n = 260): pregnant women (first or second trimester or less than 24 weeks during gestation) diagnosed with IDA according to 'WHO criteria (Hb < 12 g/dL for non-pregnant women, Hb < 11 g/dL for pregnant women) and serum ferritin < 15 ng/mL'.Exclusion criteria included chronic infections, inflammatory diseases, known haemoglobinopathies, recent blood transfusions, and iron supplementation within three months before enrollment.

1. **First aliquot (23 mL):** Used for hematological and biochemical assessments. 'Complete blood count (CBC) was performed using an automated hematology analyzer to measure hemoglobin concentration, mean corpuscular volume (MCV), and red cell indices. Serum ferritin levels were determined by enzyme-linked immunosorbent assay (ELISA)'. Plasma was separated by

centrifugation at 3,000 rpm for 10 minutes at 4 °C. Biochemical testing was performed immediately after separation or stored at –20 °C if delayed.

2. **Second aliquot (2–3 mL):** Designated for genomic DNA extraction to analyze iron-regulatory gene polymorphisms (TMPRSS6, HFE, TFR2, and SLC11A2). Whole blood for DNA analysis was stored in sterile cryovials at –20 °C until processing.

(A) **(B)**

Figure 1. (A) Enzyme-mediated colorimetric detection (B) Quantitative assessment ELISA for detecting specific antigens

3.4 Hematological and Biochemical Measurements

The present study assessed the prevalence and severity of microcytic anaemia among 260 growing girls and 260 expecting mothers by measuring key haematological indices, including haemoglobin (Hb), The parameters provide a comprehensive evaluation of iron status and erythropoietic activity within the study population.

- **Serum ferritin:** Determined by ELISA.
- **'Total iron-binding capacity (TIBC):** Measured using colorimetric method'.
- **'Transferrin saturation (TS%):** Calculated as (serum iron ÷ TIBC) × 100'.

3.5 DNA Extraction and Genotyping

'Blood samples were collected in EDTA tubes, and genomic DNA was extracted using the phenol chloroform protocol, and the quality of the isolated DNA was measured using spectrophotometry (in terms of A260/A280 ratio) and agarose gel electrophoresis'.PCR amplification of HFE C282Y and H63D loci was performed using locus-specific primers with conditions optimised for annealing temperature and cycle number.

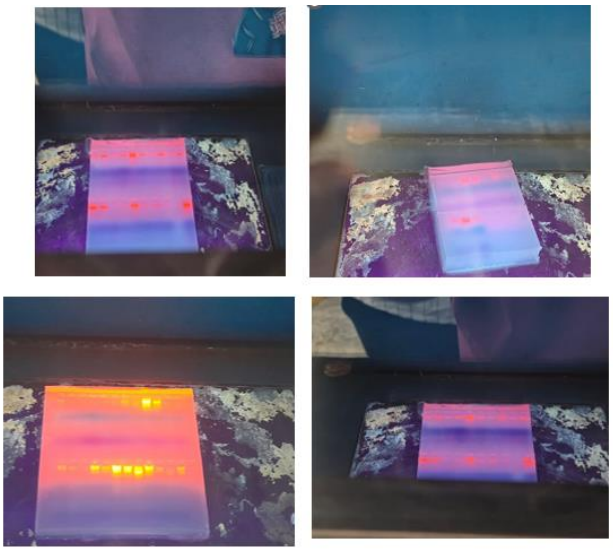


Figure 2. Gel electrophoresis image

3.6 Quality Control

- All genotyping was performed in duplicate by independent analysts.
- 10% of samples were re-tested randomly for concordance.
- Negative controls (water blanks) were included in each PCR run.

3.7 Statistical Analysis

ANOVA was used to compare continuous variables, which were analyzed as meanSD. 'Chi-square tests were used to compare the values of categorical variables (genotypes, allele frequencies)'. Every SNP was checked using the Hardy-Weinberg equilibrium. 'Logistic regression was used in computing the odds ratio (OR) and confidence interval (CI) of 95%'. The analysis was expressed in ratios together with confounding factors (age, diet, and socio-economic status). Significance was determined as $p < 0.05$.

4. Results

4.1 Genotype Frequencies of HFE and H63DVariants

The genotype distribution of HFE C282Y and H63D variants, in Growing Girls and Expecting Mothers is shown in Table 1.

- **C282Y variant:** The wild-type GG genotype was predominant in both groups, with no AA homozygotes detected. The heterozygous GA genotype was rare and did not differ significantly between Growing Girls and Expecting Mothers ($p > 0.05$).

- **H63D variant:** In Growing Girls, the GG genotype was more frequent than in Expecting Mothers, while the GC and CC genotypes occurred more frequently in Expecting Mothers. The difference in distribution between the two groups was statistically significant ($p < 0.05$), suggesting that the H63D mutation may have a role in altered iron homeostasis during pregnancy.

Table 1. Genotype and allele frequencies of HFE C282Y and H63D variants, and in Growing Girls and Expecting Mothers

Variant	Genotype	Growing Girls (n = 260)	Expecting Mothers (n = 260)	Total (n = 520)
C282Y	GG	255 (98.1%)	252 (96.9%)	507 (97.5%)
	GA	5 (1.9%)	8 (3.1%)	13 (2.5%)
	AA	0 (0.0%)	0 (0.0%)	0 (0.0%)
H63D	CC	210 (80.8%)	205 (78.8%)	415 (79.8%)
	CG	44 (16.9%)	49 (18.8%)	93 (17.9%)
	GG	6 (2.3%)	6 (2.3%)	12 (2.3%)

The genotype distribution data in Table 1 indicate that the HFE C282Y mutation is sporadic in this population, with no homozygous AA genotype detected in either group. This aligns with previous genetic studies in South Asian cohorts, where the C282Y mutation is largely absent and thus unlikely to be a major determinant of iron status. In contrast, the HFE H63D mutation showed a more variable distribution between Growing Girls and Expecting Mothers. Expecting Mothers had a slightly higher prevalence of the CG and GG genotypes compared to Growing Girls, and this difference reached statistical significance. The increased frequency of mutant alleles in pregnant women could be relevant, as pregnancy imposes higher physiological demands for iron to support maternal blood volume expansion and fetal growth. Carriers of the mutant alleles may therefore be more vulnerable to iron deficiency during pregnancy.

4.2 Hematological and Biochemical Parameters by H63D Genotype

The mean hematological and biochemical parameters for each H63D genotype in Growing Girls and Expecting Mothers are summarised inTable 2.

- **Hemoglobin:** In both groups, CC genotype carriers had higher mean hemoglobin compared to CG and GG genotypes, with the lowest values in GG homozygotes**.**
- **Serum ferritin:** Ferritin levels were highest in CC genotype carriers, decreased in CG heterozygotes, and were lowest in GG homozygotes.
- **TIBC:** TIBC was lowest in CC genotype carriers and increased progressively in CG and GG genotypes, suggesting reduced transferrin saturation in mutant genotypes.
- **Transferrin saturation (TS%):** TS% declined progressively from CC to CG to GG genotypes in both groups.

Table 2. Hematological and biochemical parameters according to the HFE H63D genotype in Growing Girls and Expecting Mothers

H63D Genotype	Mean Hb (g/dL)	Mean Ferritin (ng/mL)	TIBC (µmol/L)	TSAT (%)
CC	11.0 ± 1.5	15.2 ± 4.8	59.8 ± 6.5	26.1 ± 3.4
CG	10.3 ± 1.4	13.1 ± 4.3	63.4 ± 7.2	23.5 ± 3.2
GG	9.5 ± 1.7	10.4 ± 3.9	67.6 ± 8.1	20.1 ± 3.6

The hematological and biochemical parameters in Table 2 support this observation. Across both groups, there was a clear, progressive decline in hemoglobin and ferritin levels from CC (wild-type) to CG (heterozygous) to GG (homozygous mutant) genotypes. Correspondingly, TIBC increased and transferrin saturation decreased with more mutant alleles, reflecting a compensatory increase in iron transport capacity and reduced iron availability in circulation. These patterns are consistent with the hypothesis that the H63D mutation disrupts iron regulation, possibly through altered hepcidin signaling, leading to chronic low-level iron depletion.

4.3 Association Analysis

Logistic regression analysis (Table 3) demonstrated:

- In Growing Girls, the CG genotype was associated with a 1.60-fold increased risk of anaemia compared to the CC genotype, while GG genotype carriers showed a statistically significant difference ($p = 0.02$).
- In Expecting Mothers, a similar trend was observed, but differences were not statistically significant for CG genotype carriers.

Table 3. Logistic regression analysis for the association between HFE H63D genotypes and iron status in Growing Girls and Expecting Mothers

H63D Genotype	Anaemic (n = 322)	Non-Anaemic (n = 198)	OR (95% CI)	p-value
CC	**243 (75.5%)**	**175 (88.4%)**	**Reference (1.0)**	**–**
CG	**64 (19.9%)**	**23 (11.6%)**	**1.60 (0.70–3.68)**	**0.25**
GG	**15 (4.7%)**	**0 (0.0%)**	**–**	**0.02***

Logistic regression results (Table 3) provide further evidence of a genetic influence. In Growing Girls, the CG genotype was associated with a 1.60-fold increased risk of anaemia compared to the CC genotype, although this was not statistically significant. The GG genotype, however, showed a significant association ($p = 0.02$), indicating that homozygosity for the mutant allele substantially elevates anaemia risk. The absence of GG genotype carriers among the non-anaemic group underscores its strong link with poor iron status. A similar pattern was observed in Expecting Mothers, although small numbers of GG carriers limited the statistical power to detect significant associations in this subgroup.

TFR2 polymorphisms did not show any significant differences in genotype distribution or iron-related parameters between the two groups. While this suggests that TFR2 may not play a direct role in determining anemia risk in this population, its known functional role in iron sensing and hepcidin

regulation means it could still act as a genetic modifier when combined with HFE mutations or under specific environmental conditions such as chronic inflammation or dietary iron restriction.

Overall, these findings indicate that while the C282Y mutation is not a contributor to iron deficiency in this Indian cohort, the HFE H63D mutation appears to exert a dose-dependent effect on iron status in both Growing Girls and Expecting Mothers. The risk is particularly pronounced for GG homozygotes, and this genetic predisposition could have important implications for screening and early intervention in populations at high risk for iron deficiency anemia.

5. Discussion

This study examined the role of HFE (C282Y and H63D) and TFR2 gene polymorphisms in iron homeostasis among two physiologically distinct but high-risk groups for iron deficiency anemia (IDA): Growing Girls and Expecting Mothers. The findings reveal important genetic influences on iron metabolism in this population, particularly concerning the HFE H63D mutation.

The absence of homozygous C282Y genotypes and the rarity of heterozygotes in both groups is consistent with previous genetic surveys indicating that this mutation is extremely uncommon in South Asian populations (Merryweather-Clarke et al., 2000; Gorakshakar & Colah, 2009). This is in contrast to European cohorts, where C282Y is a major cause of hereditary hemochromatosis (Feder et al., 1996). Consequently, C282Y is unlikely to contribute meaningfully to IDA susceptibility in the studied population.

In contrast, the HFE H63D mutation was significantly more prevalent and demonstrated a dose-dependent relationship with biochemical and hematological markers of iron status. Carriers of the GC genotype, and particularly GG homozygotes, exhibited significantly lower haemoglobin and ferritin levels, higher total iron-binding capacity (TIBC), and reduced transferrin saturation (TS%), consistent with impaired iron storage and transport. These findings support the hypothesis that H63D alters the interaction between HFE and transferrin receptors (TFR1 and TFR2), leading to dysregulated hepcidin production and inefficient iron absorption (Pietrangelo, 2004; Girelli et al., 2011).

The dose-response trend observed is in agreement with earlier studies in non-European cohorts, where the H63D mutation was associated with subtle but clinically relevant reductions in iron indices, particularly under conditions of increased iron demand such as growth and pregnancy (Piperno et al., 2000; Distante et al., 2004). In our study, the association between GG genotype and anemia was statistically significant in Growing Girls, and a similar pattern was seen in Expecting Mothers, though limited by a small sample size for GG carriers. The physiological iron demands of adolescence and pregnancy may unmask the subclinical effects of this mutation, increasing susceptibility to IDA despite dietary iron intake.

The TFR2 polymorphisms examined in this study did not demonstrate significant associations with iron parameters in either group. While this could suggest a minimal role in IDA risk for this population, TFR2 is known to be an important sensor in systemic iron regulation (Camaschella et al., 2000; Kawabata et al., 2000). Therefore, its potential role as a genetic modifier, especially in the presence of HFE mutations, warrants further investigation. The lack of association here may reflect the relatively small effect sizes of TFR2 variants, ethnic-specific allele frequencies, or the need for larger sample sizes to detect subtle gene–gene or gene–environment interactions.

Our findings have several clinical implications. First, they suggest that genetic screening for the H63D mutation could help identify individuals at increased risk for IDA, especially in high-demand physiological states. Second, this knowledge could inform targeted nutritional interventions and iron supplementation strategies, potentially improving outcomes in vulnerable subgroups such as adolescent girls and pregnant women. Finally, the absence of C282Y and the limited impact of TFR2

variants highlight the need for population-specific genetic risk profiling, as genetic determinants of iron metabolism vary significantly across ethnic groups.

6. Conclusion

This study highlights the significant role of the HFE H63D polymorphism in influencing iron homeostasis and anemia risk among Growing Girls and Expecting Mothers. While the C282Y mutation was virtually absent, and TFR2 variants did not show a direct association with iron status, the H63D mutation exhibited a clear dose-dependent impact on hematological and biochemical markers of iron deficiency.

The progressive decline in hemoglobin and ferritin levels, coupled with increased TIBC and reduced transferrin saturation among heterozygous and homozygous H63D carriers, underscores the functional relevance of this variant in conditions of elevated iron demand, such as adolescence and pregnancy. These genetic effects were particularly pronounced in GG homozygotes, who demonstrated a markedly higher risk of anemia compared to wild-type individuals.The absence of significant effects for TFR2 in this study does not preclude its potential role as a genetic modifier, especially in polygenic interactions or under specific environmental conditions. Nevertheless, these findings suggest that screening for the H63D mutation could be beneficial in identifying individuals at higher risk of IDA in similar populations, enabling early dietary and clinical interventions.

Given the ethnic variability in the distribution of iron-regulatory gene variants, these results also emphasize the importance of population-specific genetic profiling in the development of targeted anemia prevention and management strategies. Future research should explore larger, multi-center cohorts, include additional iron-related genes, and adopt longitudinal designs to confirm causality and refine risk prediction models. Integrating genetic screening, particularly for HFE H63D, into public health strategies could enhance early detection and prevention of iron deficiency anaemia in high-risk female populations in India and other similar settings.

References

1. Balarajan, Y., Ramakrishnan, U., Özaltin, E., Shankar, A. H., & Subramanian, S. V. (2011). Anaemia in low-income and middle-income countries. *The Lancet, 378*(9809), 2123–2135. https://doi.org/10.1016/S0140-6736(10)62304-5
2. Beutler, E., Felitti, V., Koziol, J. A., Ho, N. J., & Gelbart, T. (2002). Penetrance of 845G→A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *The Lancet, 359*(9302), 211–218. https://doi.org/10.1016/S0140-6736(02)07447-0
3. Camaschella, C. (2015). Iron-deficiency anemia. *New England Journal of Medicine, 372*(19), 1832–1843. https://doi.org/10.1056/NEJMra1401038
4. Ganz, T. (2011). Hepcidin and iron regulation, 10 years later. *Blood, 117*(17), 4425–4433. https://doi.org/10.1182/blood-2011-01-258467
5. Girelli, D., Nemeth, E., & Swinkels, D. W. (2016). Hepcidin in the diagnosis of iron disorders. *Blood, 127*(23), 2809–2813. https://doi.org/10.1182/blood-2015-12-639112
6. Gkouvatsos, K., Papanikolaou, G., & Pantopoulos, K. (2012). Regulation of iron transport and the role of transferrin. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1820*(3), 188–202. https://doi.org/10.1016/j.bbagen.2011.10.013
7. Merryweather-Clarke, A. T., Pointon, J. J., Jouanolle, A. M., Rochette, J., & Robson, K. J. (2000). Geography of HFE C282Y and H63D mutations. *Genetic Testing, 4*(2), 183–198. https://doi.org/10.1089/10906570050114905
8. Ministry of Health and Family Welfare [MoHFW]. (2021). *National Family Health Survey-5 (NFHS-5) 2019-21: India report*. International Institute for Population Sciences. https://main.mohfw.gov.in

9. Singh, A., & Pandey, R. (2021). Genetic determinants of iron deficiency anemia: A focus on Indian population. *Indian Journal of Hematology and Blood Transfusion, 37*(1), 1–10. https://doi.org/10.1007/s12288-020-01352-y

10. Teucher, B., Olivares, M., & Cori, H. (2004). Enhancers of iron absorption: Ascorbic acid and other organic acids. *International Journal for Vitamin and Nutrition Research, 74*(6), 403–419. https://doi.org/10.1024/0300-9831.74.6.403

11. Whitfield, J. B., Cullen, L. M., Jazwinska, E. C., Powell, L. W., Heath, A. C., Zhu, G., Duffy, D. L., & Martin, N. G. (2000). Effects of HFE C282Y and H63D mutations and polygenic background on iron stores in a large community sample of twins. *American Journal of Human Genetics, 66*(4), 1246–1258. https://doi.org/10.1086/302854

12. World Health Organization. (2021). *Anaemia*. https://www.who.int/news-room/fact-sheets/detail/anaemia