Iron deficiency anemia among the children in India- An Overview

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Short Abstract:

Iron deficiency anemia, a major health problem in children with serious consequences is of great public health importance. It is attributed mainly due to poor nutritional iron intake, low iron bio-availability and genetic factors. Thus, anemia control through primary health care should be an integral part of total health care and socio-economic development of the country.

Long Abstract:

Iron deficiency anemia is a major health problem of children with adverse effects on the development of the children. It afflicts millions of people all over the world, primarily affecting women of child bearing age, pregnant women and their young children particularly in the developing countries. The World Health Organization (WHO) has estimated that, globally, 1.62 billion people are anemic, with the highest prevalence of anemia (47.4%) among preschool-aged children; of these 293 million children, 89 million live in India. The third National Family Health Survey (NFHS) 2005-2006 revealed that at least 80% of India among rural children aged between 12 to 23 months were anemic. Anemia was especially prevalent as the majority of India's population is rural(72.2%). However, despite recent economic development and the existence of a national anemia-control program, the prevalence of anemia in India is increasing at a fast pace in children aged 6 to 36 months. It is the most common type of anemia caused by inadequate iron availability for hemoglobin production due to the lack of dietary iron or insufficient uptake of iron. A number of conditions are associated with anemia, including
nutritional or absorptive deficiency, infectious diseases, blood loss and genetic mutations. The genes involved in iron metabolism such as transferrin (TF) and its receptors 1 and 2 (TFR1 and TFR2) and matriptase (TMPRSS6) are essential proteins for the uptake of iron by the cell. The present study highlights the role of these genes in iron deficiency anemia.

**Introduction:**

Iron deficiency is one of the leading risk factors for disability and mortality worldwide, affecting both developing and developed countries with major consequences for human health as well as social and economic improvement [1]. It is the most frequent nutritional problem on a worldwide scale, affecting approximately two billion people, 85% of whom suffer as a consequence of deficient iron ingestion and low absorption capacity. Thus, it can be estimated that, at present, 34% of the world’s population suffers from iron deficiency, with 80% belonging to developing countries, in which the incidence of anemia and iron deficiency is approximately 40%, whereas in developed countries, its prevalence is lower than 10% [2]. In total, 800,000 (15%) of deaths are attributed to iron deficiency. WHO lists iron deficiency (ID) as one of “Top Ten Risk Factors contributing to Death” [3]. IDA is more common in South Asian countries especially, India, Bangladesh and Pakistan. By contrast, the prevalence of IDA in neighboring countries such as Bangladesh and Pakistan has declined to 55% [4]. The reduction of IDA prevalence in China is especially remarkable i.e., the prevalence was halved from 20% to the current level of 8% within a decade [5]. It is very difficult to ascertain the true incidence of IDA, as the etiology of anemia is multifactorial. In a large scale study conducted by ICMR (Indian Council Medical Research) about 53% of children were found to be anemic [6]. A study by NFHS 2002 [7] found that anemia prevalence among children aged 1–5 years is little lower than pre-school children, adolescents and women of child bearing age who are at risk of developing anemia [8]. IDA is an extremely serious public health problem in India especially among pregnant and lactating women, children and adolescents [9]. Over the last 50 years, the prevalence of iron deficiency anemia has ranged between 68 to 97 percent in the children. [10,11].
Iron Absorption and Balance:

Plasma and cellular iron levels are tightly regulated by mechanisms that control iron absorption, storage, recycling and release. To absorb iron, the insoluble ferric iron (Fe³⁺) from vegetables and grains needs to be converted into the ferrous form (Fe²⁺) by a brush border ferric reductase in the duodenum and upper jejunum. The ferrous iron is then transferred across the enterocyte membrane by divalent metal transporter (DMT) 1. In blood, iron is carried by transferrin to various tissues to be taken by cells in a transferrin receptor-mediated endocytotic process[12]. Once inside the cell, iron is released from transferrin in the acidic endosome and stored in the form of ferritin. A significant portion, up to ~75%, of plasma iron is taken up by bone marrow to synthesize hemoglobin in red blood precursors. Several other cell types including enterocytes, hepatocytes, and reticuloendothelial macrophages also serve as major iron storage sites. When plasma iron levels are low, iron release is increased from enterocytes, hepatocytes and macrophages to meet the physiological demand. Conversely, when plasma iron levels are high, iron will remain stored in these cells. The body loses iron when cells are shed from the gastrointestinal tract or blood during hemodialysis [13].

Iron Deficiency Anemia, Infants and Children:

Iron deficiency is common in children, largely because of the demands for a positive iron balance that is imposed by growth and partly because diets rich in milk are not a good source of iron. Adverse effects of iron deficiency on behavioral patterns are of special concern in the infants because the later part of the brain growth spurt coincides with the period in which iron deficiency anemia is most prevalent in the age group between 6–24 months. Earlier studies have suggested that iron deficient children have lower IQ scores, decreased attentiveness and lower scores on tests of academic performance compared with non-anemic controls. However, a cause and effect relationship could not be established because of associated multiple confounding factors in most of the studies including low socioeconomic status, poverty, lack of stimulation at home, poor parental education, maternal depression, low birth weight, faulty feeding, malnutrition, parasitic infestations and elevated lead levels. Several studies have
suggested that iron deficiency in infancy may be associated with impaired cognitive function during the school years [14].

Iron is essential for all tissues in a young child’s developing body. Presence of iron in the brain from early life is essential as it participates in the neural myelination processes, along with growth and immune function [15]. Iron, which is essential to both the host and invading pathogens, must be carefully regulated to promote optimal conditions that preserve the health of young children. Furthermore, iron can interfere with the absorption of other nutrients and, in excess, can generate free radicals that impair cellular functions and suppress enzymatic activity [16]. Observational studies in children over 2 years have reported poorer cognition and school achievement in iron deficient children [17]. Adolescent girls are particularly susceptible to iron deficiency because of poor dietary intake along with increased iron requirement related to rapid growth and menstrual blood loss and are at greater risk of cognitive impairment. In therapeutic trials, few randomized controlled trials are available, as withholding iron to iron deficient children was considered unethical. Hence, most of the studies have no controls. Pollitt et al (1985) studied children with iron deficiency anemia where mean age was 9.5 years. Oral ferrous sulphate 50 mg daily was given in this randomized controlled trial, wherein a significant improvement in mental scores in iron treated anemic children compared to placebo treated anemic children was observed [18]. Further efficiency scores of iron treated anemic children became similar to that of non-anemic children.

**Role of genes in Iron Metabolism:**

Iron is an essential element required for the growth and survival of most organisms, and the iron balance is therefore tightly regulated by several interacting iron binding factors [19]. Heritable differences in the expression of the genes like transferrin, transferring receptors, matriptase-2, hepcidin seems to exist which may determine the phenotypic variation in iron metabolism between individuals.

**Transferrin in Iron Metabolism and Anemia:**

Serum transferrin plays a crucial role in iron metabolism as it provides most of the iron required for various functions of an organism [20]. Cells obtain iron from plasma
where it circulates in a complex with a carrier protein transferrin (Tf). Iron loaded Tf is then bound to transferrin receptor (TfR), and their complex passes into cells by means of internalization, wherein iron releases by pH-dependent mechanism [21].

Transferrin receptor is a transmembrane protein that participates in iron transport from plasma into cells. It consists of two identical subunits of 95 kDa linked by two disulfide bonds. Each TfR subunit contains an N-terminal cytoplasmic domain (1-67 amino acid residues), a transmembrane domain (68-88 amino acid residues) and a C-terminal extracellular domain (89-760 amino acid residues) [22]. The main pool of TfR molecules is located on erythroblasts which demand a lot of iron for hemoglobin synthesis. After the erythroid cells have matured, the extracellular part of the TfR molecule is truncated from the cell surface by cleavage of an R100 – L101 bond. TfR released into the blood stream consists of 101-760 amino acid residues of cell TfR and is called soluble (or serum) transferrin receptor (sTfR) [23]. The expression of transferrin receptor depends on the concentration of iron in the cellular cytoplasm. The concentration of soluble transferrin receptor (sTfR) has been reported to be proportional to the total amount of cell-associated transferrin receptor. In blood, soluble TfR is completely bound to Tf and circulates as sTfR-Tf complex. Transferrin receptor is the main receptor for transferrin and allows transferrin-bound iron uptake by the cell. Its expression is regulated by cellular iron requirements. Conserved iron-response elements in the 3'-untranslated region of transferrin receptor mRNA enhances binding of iron regulatory proteins 1 and 2. The hereditary hemochromatosis protein HFE competes binding with transferrin for an overlapping binding site. It is also involved in materno-fetal iron transport via the placenta.

The determination of the sTfR level in blood has become widely used in clinical practice [24]. The normal concentration of sTfR in blood ranges within 2 – 5 µg/ml. An increase in the sTfR level was found in iron deficiency anemia, autoimmune hemolytic anemia, hereditary spherocytosis, β-thalassemia, sickle cell anemia etc. Soluble TfR is indispensable marker of iron deficiency anemia and is mainly used for the differentiation between iron deficiency anemia (accompanied by an increase in the sTfR level) and anemia of chronic disease (proceeded at the normal sTfR level) [25]. The measurement of
Tf is also widely used in diagnosis of anemia together with the determination of sTfR, ferritin and iron concentration in serum. Because the synthesis of TfR is upregulated with tissue iron deficiency, IDA can be identified readily by an elevated serum TfR Importantly, the serum TfR is normal in anemic and chronic diseased individuals but gets elevated if they develop IDA [26].

There are no disease-causing mutations in the TFRC gene. However, missense coding region variants that may have functional effects was observed. The only one with appreciable frequency (rs3817672) is in exon 4 and encodes S142G amino acid substitution, which is ethnic/geographic influenced. The minor allele of the gene in Caucasians is the major allele in Asians and Africans. Tfrc knockout mice are not viable and die during embryonic development due to erythropoietic and neuronal development abnormalities. The short arm of chromosome 3 also harbors other iron-related genes: transferrin (3q22.1), lactotransferrin (3q21-q23), melanotransferrin (3q28-q29) and ceruloplasmin (3q23-q25), which as a gene family may be involved in compound heterozygotes in anemic individuals [27].

**Matriptase/TMPRSS6 in Iron Metabolism and Anemia:**

Matriptase-2, also called TMPRSS6, is a type II transmembrane serine protease [28]. The 811-amino-acid (aa) human protein is synthesized as an inactive zymogen and autoactivated by proteolytic cleavage. Structurally, matriptase-2 contains a short 54-aa N-terminal cytoplasmic domain, a membrane-spanning region, an SEA (sea urchin sperm protein, enteropeptidase, and agrin) domain, 2 CUB [Cls/Clr, urchin embryonic growth factor, and bone morphogeneic protein (BMP)-1] domains, three LDLa (low-density-lipoprotein receptor, class A) domains, and a trypsin-like serine protease domain containing the catalytic triad of histidine, aspartate, and serine residues.

Recently, *TMPRSS6* has been identified as a modifier of iron homeostasis because it regulates the expression of the systemic iron regulatory hormone hepcidin [29] and inhibits hepcidin activation by cleaving membrane hemojuvelin [30]. Hepcidin controls iron absorption by binding to the only known cellular iron export protein ferroportin thereby leading to ferroportin degradation and blockage of iron entering from the
enterocyte into the circulation[31]. In addition, hepcidin blocks the transfer of iron from macrophages into the circulation, which is the major iron source for erythropoiesis following erythrophagocytosis and re-utilization of the metal from senescent erythrocytes [32, 33]. In iron deficiency, however, low iron levels inhibit hepcidin formation and thus enable iron to be transferred from the gut to the blood. Part of the iron-mediated control of hepcidin can be referred to the action of TMPRSS6 and thus functional mutations in this gene are associated with insufficient iron absorption and increased hepcidin levels [34]. A recent study by Falco et al, (2010) have shown novel mutations in TMPRSS6 gene predicted to result in truncated protein lacking the catalytic domain associated with iron-refractory iron deficiency anemia (IRIDA) [35]. In recent years, association and genome-wide association (GWA) studies of this gene has revealed various mutations and furthered our understanding of iron metabolism.

Currently, there is a limited understanding of genetically acquired iron-limited anemias. In accordance with its role in co-ordinating body iron levels, alterations in the genes encoding hepcidin or its key regulators induce iron overload syndromes such as hereditary hemochromatosis (HH). Consistently, HH disorders result from inadequate hepcidin production relative to body iron stores [36]. Conversely, elevated hepcidin levels have been described in iron deficiency anemia patients that are insensitive to oral iron therapy and display an incomplete hematologic recovery with parenteral iron administrations, a condition termed iron refractory iron deficiency anemia (IRIDA) [37]. Heterozygous and homozygous biallelic human matriptase-2 mutations have been identified by 3 independent studies in 14 IRIDA patients from Northern European, African and Afro-American, Mediterranean and English ancestries [38]. Predominately identified mutations like 1906_1907insGC, 1813delG, IVS13+1G>A, IVS15-1G>C, 1383delA, IVS6+1G>C, 1179T>G and 1795C>T, encode for matriptase-2 proteins which lack functional protease domains. Associations between SNP in TMPRSS6 gene on chromosome 22q12 variants and hemoglobin levels were found in individuals with Indian Asian ancestry as well as in those with European ancestry [39, 40]. Overexpression of the protease domain deficient mask human matriptase-2 in zebrafish resulted in reduced hemoglobinization in comparison to wild-type human matriptase-2, illustrating the in vivo impact a deficiency in matriptase-2 proteolytic activity [41]. In fact, a splicing error
in Tmprss6 has been detected in \textit{Mask} mice, which have a recessive chemically, induced phenotype characterized by progressive loss of body hair and severe iron deficiency due to reduced absorption of iron from the gastrointestinal tract. \textit{Mask} mice produce a truncated copy of the Tmprss6 protein lacking the serine protease domain and express inappropriately high levels of hepcidin. These high levels can be responsible for iron deficiency and severe microcytic anemia was previously observed in transgenic mice over expressing hepcidin [42]. Collectively these data suggest that further studies of matriptase-2 gene may contribute to a better understanding of the functional relevance of matriptase-2 in regulating iron homeostasis and to translate this information into clinical answers for patients with IRIDA or other deficiencies in iron metabolism.

**Hepcidin in Iron Metabolism and Anemia:**

Hepcidin, a small acute phase antimicrobial peptide appears to synchronously orchestrate the response of iron transporter and regulatory genes to ensure proper balance between dietary iron absorption by the small intestine or circulatory hepcidin released by macrophages [43]. Hepcidin, encoded by HAMP gene, is a recently discovered 25 amino acid peptide that, in addition to being involved in innate immunity, appears to play a crucial role in iron homeostasis in humans, regulating both iron absorption from the intestine and recycling by macrophages [44].

Anemia induced by mutations in mice that restrict the uptake of iron in the small intestine (sla and mk mice) showed markedly decreased hepatic hepcidin mRNA. Anemia induced by phlebotomy, or by hemolysis from phenylhydrazine, also suppressed hepatic hepcidin mRNA. Importantly, the suppressive effect of hemolytic anemia was seen even in iron-overloaded mice, suggesting that the suppression of hepcidin by anemia is a stronger effect than the stimulation of hepcidin by iron overload [45]. Several studies suggest that there are strong genetic components that underlie hepcidin regulation beyond the usual suspects (i.e. infection, inflammation, erythropoiesis, hypoxia and iron), in a manner that could impinge on phenotypic differences in susceptibility to iron-overload or anemia. Based on variation in hepcidin expression phenotypes, new emerging data suggest that heritable regulatory polymorphisms within the promoter may be linked to diseases of iron metabolism.
A CCAAT-enhancer-binding protein (C/EBP) recognition site within the hepcidin promoter provided the first evidence for cis-acting regulation of its expression by C/EBPα. [46] In support of the contribution of regulatory SNPs in hepcidin expression variation and iron metabolism, Island et al.(2009) found a C>T polymorphism in one of two bone morphogenetic protein response elements, BMP-RE, (GGCGCC→G GTGCC) in the promoter that impaired transcription of the gene, its IL-6-responsiveness and binding by Smads [47]. Similarly, Andreani et al (2009) found association between a −582A>G polymorphism in the hepcidin promoter and iron overload in thalassemia major [48]. Porto et al (2005) previously reported a SNP (G to A substitution) in the 5'UTR of the human hepcidin gene which correlated with severe hemochromatosis [49].

A very recent study by An et al (2012) in a Chinese population identified that TMPRSS6 polymorphisms were not only associated with lower serum iron and hemoglobin levels, but are also genetic risk factors for iron deficiency and IDA. They hypothesized that the altered inhibitory effect of matriptase-2 on hepcidin secretion was possibly the underlying mechanism of the increased risk of iron deficiency and IDA. SNPs in TF and TFR2, although associated with several important iron traits, did not show significant effects on iron deficiency or anemia risk [50].

Conclusions:

Currently, the precise underlying mechanisms of IDA are undefined. Considering the complexity of the regulatory network required to maintain iron homeostasis, collaborative efforts will be required to further dissect the risk factors for initiation and progression of disease. In conclusion, genetic variations in these genes may lead to differences in iron metabolism and modulate the development of iron deficiency anemia. Thus, it is necessary to reduce iron deficiency anemia through primary health care which is an integral part of total health care and socio-economic development of the country.
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