Symposium on Nutritional Anemia

# Iron Deficiency and Infection

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## ABSTRACT

Iron deficiency is the most common micronutrient deficiency in the world. Children, particularly infants living in developing countries are highly vulnerable to infectious diseases. Therefore, understanding the relationship between iron deficiency and infection is of great importance. Iron deficiency is associated with impairment of innate (natural) immunity and cell mediated immunity, thereby contributing to increased risk of infections. The iron acquisition by the microbes and their virulence is determined by various host and microbial mechanisms. Altering these mechanisms might provide modes of future therapy for infectious diseases. **[Indian J Pediatr 2010; 77 (7) : 789-793]** *E-mail: vmpd05@yahoo.co.in*

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Protein-calorie malnutrition (PCM) is associated with a significant impairment of cell mediated immunity, phagocyte function, complement system, secretary immunoglobulin A concentrations, cytokine production and an altered immune response.[1] Deficiency of various essential vitamins and minerals such as iron, zinc, selenium, copper, vitamins A, C, E and pyridoxine, and folic acid individually have been shown to have important influences on immune responses and risk of infection. Iron deficiency is associated with impairment of cell mediated immunity and the bactericidal activity of neutrophils, thereby increasing the susceptibility to infections.[2,3] Iron deficiency might play an important role in defense mechanism and thus, the term "nutritional immunity" was coined to highlight the importance of iron deficiency to prevent bacterial growth.

## IRON DEFICIENCY AND IMMUNITY

Iron is a fundamental element for normal development of immune system. Iron is essential for proper cell differentiation and cell growth. It is an important component of peroxide-generating enzymes and nitrous oxide-generating enzymes that are critical for proper enzymatic functioning of immune cells.[5] It is also involved in regulation of cytokine production and action as well as in the development of cell mediated immunity. Spear and Sherman demonstrated that iron is an integral component of enzyme myeloperoxidase (MPO), which produces reactive oxygen intermediates responsible for intracellular killing of pathogens.[6] Humoral and cell-mediated immunity both have been studied extensively, mainly *in vitro*, in relation to iron deficiency in both humans and animals. Impairment of cell mediated immunity have been well described in iron-deficient humans, however, little evidence exists for major humoral deficiencies. Various abnormalities of cellular defenses observed in iron deficiency include:[6-8]

(a) Reduced neutrophil function with decreased myeloperoxidase (MPO) activity
(b) Impaired bactericidal activity
(c) Depression of T-lymphocyte numbers with thymic atrophy
(d) Defective T lymphocyte-induced proliferative response
(e) Impaired natural killer cell activity
(f) Impaired interleukin-2 production by lymphocytes
(g) Reduced production of macrophage migration inhibition factor
(h) Reversible impairment of delayed cutaneous hypersensitivity including tuberculin reactivity.

Decreased MPO activity gets reversed on correcting iron deficiency. The humoral immunity *i.e.,* antibody production in response to immunization with most antigens, in animal studies and humans is preserved.[6,9-11] Neutrophil and macrophage dysfunction has been associated with low iron levels, as evidenced by deficient nitroblue tetrazoleum reduction and hydrogen peroxide formation in these respective cell lines.[8] Ribonucleotide

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reductase activity has been discovered to be iron dependent. Iron levels have also been shown to alter the proliferation of T helper (Th)-1 and Th-2 subsets, likely related to the difference in dependence of cells on transferrin-related iron uptake. Th-2 clones possess larger pools of iron susceptible to chelation, as compared with Th-1 cells, making Th-1 immune pathways more susceptible to changes in ambient iron concentrations. Few studies have reported decreased risk of infection (especially malaria) during iron deficiency.[12-13] The mechanisms of resistance against infections such as antibacterial properties of tissue fluids and phagocytic abilities of tissues require a virtual iron free environment to function properly.[14] High association constant of transferrin for $Fe^{3+}$ ensures that the amount of free ferric iron in plasma is about $10^{-18}$ M, which can be regarded as virtually zero. However, pathogenic organisms have several ways of extracting iron from much lower iron levels. Two well designed case control studies demonstrated impaired immune function in iron deficiency. Ahluwalia *et al* (2004) showed reduced T cell proliferation in response to stimulation with concanavalin A (Con A) and phytohemagglutinin (PHA), and less oxidative burst and bactericidal capacity in iron deficient older women.[15] However, phagocytosis and number of granulocytes expressing respiratory burst were not affected. Ekij *et al* (2005) showed significantly reduced interleukin-6 levels, immunoglobulin G levels, oxidative burst activity of neutrophils and monocytes, and phagocytic activity of monocytes in 6-24 months children.[16] The major limitation of both these studies is small number of subjects.

In summary, iron deficiency depresses certain aspects of cell-mediated immunity and innate immunity but the significance of hypoferremia (as opposed to normal transferrin saturation) on growth of microorganisms remains uncertain. In contrast, one group of intracellular organisms, Plasmodia, may have a specific disadvantage in iron deficiency. Because there are some conflicting effects of iron deficiency on defense systems, it becomes more important to review the relationship between iron deficiency and infection risk.

## IRON DEFICIENCY AND INFECTION

Murray *et al* (1978) noted that Somali nomads entering a feeding camp had no infections if they were iron deficient, in contrast to a high rate of infection in those with normal iron status.[12] In contrast, Snow and colleagues (1991) did not find a significant correlation between measures of iron status and subsequent malarial experience in 1 to 9 year old Gambian children.[17] Oppenheimer and his colleagues in 1986 observed that infants with lower hemoglobin values at birth were less likely to have malaria at field follow-up and less likely to be admitted to hospital during first year of life.[13] Because birth hemoglobin is the main iron source during the first year, this association may mean that iron deficiency protects from malaria and other infections. There is a possibility that this protective effect on malarial infection in these infants may be due to homozygous single gene deletion α-thalassemia, which is present in >50% of this population. Indeed the high prevalence of single-deletion α-thalassemia in many tropical areas may have a confounding effect in many studies of iron, anemia and morbidity because the mutation causes both anemia and protection against malaria and other infections.[18] Only few observational clinical studies in iron-deficient humans convincingly relate such deficiency to substantial morbidity due to infections. Higgs and Wells (1973) noted that of 31 patients with chronic mucocutaneous candidiasis, 23 were iron deficient and 9 of 11 improved with oral and parenteral iron therapy alone, with a regression of oral lesions and development of delayed hypersensitivity to Candida.[19] In an another report, 16 patients with recurrent staphylococcal furunculosis also had nonanemic iron deficiency. Furunculosis resolved after 3–4 wk of iron therapy in all but one patient.[20] In a prospective study by Harju and his colleagues, postoperative infections after abdominal surgery were reported to be significantly more common in 228 patients with low preoperative serum ferritin compared with 220 patients with normal ferritin.[21] Therefore, several studies indicate that iron deficiency is a risk factor for infections with the exception of malaria, where it might confer protection.

## IRON SUPPLEMENTATION AND INFECTION

Earlier studies conducted in deprived populations of temperate, developed countries tended to support the value of iron supplements in reducing rates of respiratory infections in infants.[22-24] Parenteral iron (iron dextran) administration at birth, and during early infancy resulted in increased infections in neonates.[25] Oral iron supplementation in adult nomads has also resulted in increased attacks of malaria.[12] However, in a prospective controlled trial of parentral iron dextran in premature infants, no difference in control or intervention group was observed over one yr.[26] Similarly, in a randomized trial, daily oral iron supplementation given to 6-36 month old Togolese children during malaria transmission season was also found to have no impact on the incidence of infections, especially malaria.[27] In a review by Gera *et al* (2002), authors concluded that iron supplementation has no apparent harmful effect on the overall incidence of infectious illnesses in children.[28]

Two well designed recent trials of sufficient large size have evaluated the prevalence of infections during iron supplementation in Tanzania and Nepal. Tanzania study compared iron+folic acid ($n$=7950), iron+folic acid +zinc

(*n*=8120), and placebo (*n*=8006) given to children aged 1 to 35 months and was designed with adequate power to detect the effects of iron-folic acid (IFA) supplementation on mortality.[29] Although the trial arms administering IFA were discontinued because of increased mortality (relative risk 1.61, 95% confidence interval 1.03–2·52), serious adverse events (relative risk 1.32, 95% CI 1.10–1.59), and hospital admissions (relative risk 1.28, 95% CI 1.05–1.55) secondary to malaria in the IFA group, post hoc analysis revealed that iron deficient, anemic children given IFA were actually protected from malaria related events compared with placebo (relative risk 0.56, 95% CI 0.32–0·97), thus suggesting iron deficiency anemia to be a risk factor even for malarial morbidity and mortality. A simultaneous study of similar design and sample size was conducted in Southern Nepal, a non-malaria endemic region.[30] This study identified no evidence of harm from IFA supplementation in terms of mortality, despite having 29,097 child years follow up.

Based on the evidence reviewed, it is believed that oral iron supplementation has not been shown to cause an increased risk of infection in any age group in non-malaria endemic regions. Older reports suggest that infectious morbidity could be markedly reduced with iron supplementation.[22-24] Iron supplementation promotes production of free radicals, and this may have a deleterious effect on the immunity of a child. Ironically, defenses against free radicals are compromised in iron deficiency and malnutrition, which co-exist in the developing countries. However, iron supplementation in malaria-endemic regions may carry significantly increased risk of clinical malaria if given in therapeutic doses at times of malaria transmission.

### Mechanisms Determining Iron Acquisition by Pathogenic Organisms[8,14]

The morbidity and mortality following infectious diseases is determined by acquisition of iron by the microbes. The net iron acquisition by the microbes is determined by the balance between microbial mechanisms to acquire iron and host defense system to deprive the microbes of iron.

Pathogenic organisms have developed several ways of extracting iron from low host iron environment. These are:

- Secretion of siderophores (very high affinity for $Fe^{3+}$), which compete with transferrin for iron and transport iron to the bacteria under normal physiological conditions (*e.g., E. coli, Klebsiella, Salmonella, Pseudomonas* and *Candida species*).
- Expression of transferrin or lactoferrin receptors, which can remove iron directly from transferrin or lactoferrin (*e.g., Haemophilus influenzae, Neisseria meningitidis, Neisseria gonorrhoeae, Helicobacter pylori*). Transferrin using bacteria are found typically in plasma and cerebrospinal fluid, whereas lactoferrin using bacteria (*e.g., Helicobacter pylori*) are found on mucosal surfaces.
- Heme receptor transporter and heme-specific protein transport system, which transports heme into the bacterial cytoplasm (*e.g., Yersinia enterocolitica, Yersinia pestis, E. coli, Vibrio cholerae, H. influenza*), provides advantage to the microbes in the presence of free heme liberated after trauma or disease. Thus, heme-binding proteins (haptoglobin and hemopexin) can be bacteriostatic and interfere with microbial acquisition of iron.
- Methaemalbumen, produced from liberation and oxidation of hemoglobin, is an effective source of iron for Staphylococci.
- Plasmodium and Bartonella species invade erythrocytes to acquire iron.
- Candida species are able to bind and lyse erythrocytes to acquire intracellular iron stores.

## MECHANISMS DEVELOPED BY THE HOST TO DEPRIVE MICROORGANISMS OF IRON[8,31-34]

With the exception of *Borrelia burgdorferi* and nonpathogenic *lactobacilli*, all groups of protozoa, fungi and bacteria require iron for survival and replication. The host has developed its own mechanisms to deprive microbes of iron.

### 1. Constitutive Mechanisms

It includes ferritin inside the cells; transferrin in plasma, lymph and cerebrospinal fluid; lactoferrin in secretions of mammary glands and of respiratory and gastrointestinal tracts.

- **Lactoferrin**

Lactoferrin is a prominent iron-binding protein and immune modulator. It functions as a first line defense against invading organisms through its ability to sequester iron. Furthermore, the lactoferrin sequence comprises a defensin-like peptide, lactoferricin, which has microbicidal activity against *Candida albicans*, *Streptococcus mutans*, *Vibrio cholerae* and various enterobacteria. Two factors contribute to lactoferrin's efficiency in iron sequestration in reticuloendothelial system. First, because of its higher affinity for iron at lower than physiological pH, it binds more iron than transferrin at the sites of inflammation (sites of low pH). Second, lactoferrin receptors, present on the surface of macrophages, are not expressed on the surface of erythroid precursors. During inflammation, activated macrophages exhibit an increase in the concentration of lactoferrin receptors on their surface, which further increases the iron internalization thereby reducing the iron available for microorganisms and erythroid precursors.

• **Ferritin**

A ferritin molecule can store up to 4,500 iron atoms. During inflammation, ferritin synthesis is increased under the influence of interleukin-1 and tumor necrosis factor. High ferritin synthesis and increased lactoferrin delivery of iron to macrophages play an important role in iron sequesteration.

• **Transferrin**

Transferin is an iron binding protein present in blood. However, its affinity with iron is pH dependent, making it ineffective in the presence of acidosis.

**2. Mechanisms activated during infection or inflammation**

- Interleukin-6 (IL-6), an inflammatory cytokine induces hepcidin synthesis. Hepcidin binds with ferroportin (FPN), which induces FPN internalization and degradation. The egress of iron from macrophages and enterocytes is caused by FPN, the sole iron exporter identified in mammals. Thus increased hepcidin levels through degradation of FPN, limits the egress of the iron from intestinal and macrophage cells, and results in hypoferremia.
- Downregulation of FPN gene transcription also contributes to hypoferremia.
- Activation of ferritin gene transcription.
- Apolactoferrin released from neutrophils removes iron from sites of invasion.
- Increased hepatic synthesis of haptoglobin and hemopexin which bind extracellular hemoglobin and hemin.
- There is increased synthesis of lipocalins (binds siderophores of microbes and inactivates them), nitric oxide (disrupts iron metabolism of microbes) and natural resistance associated macrophage proteins (NRAMP) by macrophages. NRAMP1 withholds iron, thereby preventing iron uptake and its utilization by microbes. NRAMP 1 targets the membrane of microbe-containing phagosomes in macrophages and monocytes.
- B lymphocytes start producing immunoglobulins against microbial cell surface receptors, which prevent iron uptake by microbes.

**3. Failure of iron withholding defense mechanisms**

This can occur due to

- Overloading of defense components with iron.
- Damage to iron containing host cells which can create free iron pool for microbes.
- Administrating drugs such as desferioxamine, deferiprone, and deferasirox which can supply iron to microbes to facilitate the microbial growth.

## FUTURE IMPLICATIONS

In view of declining effectiveness of antibiotics against microbes, we might need a different modality of antimicrobial treatment in future. Altering the mucosal micronutrient environment by manipulating host iron absorption or microbial iron use provides theoretical modes of future therapy for infectious diseases. Hepcidin plays a central role in the maintenance of iron homeostasis by inhibiting egress of iron out of macrophages and enterocytes, thus development of agonists and antagonists of hepcidin will be of significant interest. Anti IL-6 treatment (tocilizumab), which could alleviate IL-6 mediated pathology in a variety of inflammatory disorders, is already undergoing clinical trials.[35,36] Further experimental and clinical trials are needed to determine the appropriate levels of ambient iron needed to keep the host immune system at peak function, while at the same time, preventing microbial proliferation.

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## REFERENCES

1. Cunningham-Rundles S. Effects of nutritional status on immunological function. *Am J Clin Nutr* 1982; 35: S1202-S1210.
2. Chandra RK. Reduced bactericidal capacity of polymorphs in iron deficiency. *Arch Dis Child* 1973; 48: 864-866.
3. Bhaskaram C, Reddy V. Cell mediated immunity in iron- and vitamin- deficient children. *Br Med J* 1975; 3: 522-522.
4. Kochan I. The role of iron in bacterial infections with special consideration of host-tubercle bacillus interaction. *Curr Top Microbiol Immunol* 1973; 60: 1-30.
5. Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr* 2001; 131: 568S-580S.
6. Spear AT, Sherman AR. Iron deficiency alters DMBA-induced tumor burden and natural killer cell cytotoxicity in rats. *J Nutr* 1992; 122: 46-55.
7. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. *J Nutr* 2001; 131: 616S-635S.
8. Markel TA, Crisotomo PR, Wang M *et al*. The struggle for iron: gastrointestinal microbes modulate the host immune response during infection. *J Leukoc Biol* 2007; 81: 393-400.
9. Hallquist NA, McNeil LK, Lockwood JF, Sherman AR. Maternal-iron-deficiency effects on peritoneal macrophage and peritoneal natural-killer-cell cytotoxicity in rat pups. *Am J Clin Nutr* 1992; 55: 741-746.
10. Bagchi K, Mohanram M, Reddy V. Humoral immune response in children with iron-deficiency anaemia. *Br Med J* 1980; 280: 1249-1251.
11. Svoboda M, Nechvatalova K, Krejci J, Drabek J, Ficek R, Faldyna M. The absence of iron deficiency effect on the humoral immune response of piglets to tetanus toxoid. *Veterinarni Medicina* 2007; 52: 179-185.
12. Murray MJ, Murray AB, Murray MB, Murray CJ. The adverse effect of iron repletion on the course of certain infections. *Br*

*Med J* 1978; 2: 1113-1115.
13. Oppenheimer SJ, Gibson FD, Macfarlane SB *et al.* Iron supplementation increases prevalence and effects of malaria: report on clinical studies in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1986; 80: 603-612.
14. Bullen J, Griffiths E, Rogers H, Ward G. Sepsis: the critical role of iron. *Microbes Infect* 2000; 2: 409-415.
15. Ahluwalia N, Sun J, Krause D, Mastro A, Handte G. Immune function is impaired in iron-deficient, homebound, older women. *Am J Clin Nutr* 2004; 79: 516-521.
16. Ekiz C, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of immune system. *Hematol J* 2005; 5: 579-583.
17. Snow RW, Byass P, Shenton FC, Greenwood BM. The relationship between anthropometric measurements and measurements of iron status and susceptibility to malaria in Gambian children. *Trans R Soc Trop Med Hyg* 1991; 85: 584-589.
18. Oppenheimer SJ, Hill AV, Gibson FD, Macfarlane SB, Moody JB, Pringle J. The interaction of alpha thalassaemia with malaria. *Trans R Soc Trop Med Hyg* 1987; 81: 322-326.
19. Higgs JM, Wells RS. Chronic muco-cutaneous candidiasis: new approaches to treatment. *Br J Dermatol* 1973; 89: 179-190.
20. Weijmer MC, Neering H, Welten C. Preliminary report: furunculosis and hyporferraemia. *Lancet* 1990; 336: 464-466.
21. Harju E. Empty iron stores as a significant risk factor in abdominal surgery. *J Parenter Enteral Nutr* 1988; 12: 282-285.
22. MacKay HM. Anaemia in infancy; its prevalence and prevention. *Arch Dis Child* 1928; 3: 117-147.
23. Andelman MB, Sered BR. Utilization of dietary iron by term infants. A study of 1,048 infants from a low socioeconomic population. *Am J Dis Child* 1966; 111: 45-55.
24. Cantwell RJ. Iron deficiency anemia of infancy: some clinical principles illustrated by the response of Maori infants to neonatal parenteral iron administration. *Clin Pediatr* 1972; 11: 443-449.
25. Barry DM, Reeve AW. Increased incidence of gram-negative neonatal sepsis with intramuscular iron administration. *Pediatrics* 1977; 60: 908-912.
26. James JA, Combes M. Iron deficiency in the premature infant: Significance and prevention by the intramuscular administration of iron-dextran. *Pediatrics* 1960; 26: 368-374.
27. Berger J, Dyck JL, Galan P *et al.* Effect of daily iron supplementation on iron status, cell-mediated immunity, and incidence of infections in 6-36 month old Togolese children. *Eur J Clin Nutr* 2000; 54: 29-35.
28. Gera T, Sachdev HP. Effect of iron supplementation on incidence of infectious illness in children: systematic review. *Br Med J* 2002; 325: 1142-1144.
29. Tielsch JM, Khatry S, Stoltzfus RJ *et al.* Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. *Lancet* 2006; 367: 144-152.
30. Sazawal S, Black RE, Ramsan M *et al.* Effect of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised placebo-controlled trial. *Lancet* 2006; 367: 133-143.
31. Weinberg ED. Iron availability and infection. *Biochim Biophys Acta* 2009; 1790: 600-605.
32. Beaumont C, Delaby C. Recycling iron in normal and pathological states. *Semin Hematol* 2009; 46: 328-338.
33. Ganz T, Nemeth E. Iron sequestration and anemia of inflammation. *Semin Hematol* 2009; 46: 387-393.
34. Jurado RL. Iron, infections, and anemia of inflammation. *Clin Infect Dis* 1997; 25: 888-895.
35. Nishimoto N, Kanakura Y, Aozasa K *et al.* Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. *Blood* 2005; 106: 2627-2632.
36. Kawabata H, Tomosugi N, Kanda J, Tanaka Y, Yoshizaki K, Uchiyama T. Anti-interleukin 6 receptor antibody tocilizumab reduces the level of serum hepcidin in patients with multicentric Castleman's disease. *Haematologica* 2007; 92: 857-858.