Introduction

Historically (1500 B.C.), in Ayurvedic literature, Charak samhita described fatigue and pallor caused by “bloodlessness”, which can be cured by Lauha bhasma (calcified iron). During the same period, the Egyptian manual of therapeutics ‘Ebers Paprus’ described a disease characterized by pallor, dyspnea and oedema. In Greek literature (1554 – 1700) “Chlorosis/Demeorbo Virgineo” or green sickness was described as curable by drinking iron rust dissolved in water or wine.

In India, in 1968, Dr Gopalan constituted an Expert Committee of the Nutrition Society of India, to suggest measures to control anaemia in the country. The Committee, on the basis of the available data on prevalence and severity of anaemia from Delhi, Calcutta, Madras and Vellore, recommended the setting up of a National Nutritional Anaemia Prophylaxis Programme (NNAPP) for pregnant women as they were at higher risk of developing anaemia, possibly severe anaemia. The effort was a national commitment to prevent and control anaemia. Unfortunately, apathy towards the programme and disinterest in controlling iron deficiency made it a difficult journey to prevent nutritional anaemia and iron deficiency in the country.

Current knowledge in the development of iron deficiency

Iron deficiency is an end result of a long period of negative iron balance, mainly due to poor dietary availability, rapid growth of the person, and blood loss. The pathological stages are;

- **Pre-latent deficiency**: Liver (Hepatocytes and macrophages), spleen and bone marrow show reduced iron stores (reduced- bone marrow iron and serum ferritin).
- **Latent deficiency**: With very low or absent bone marrow iron stores there is progressive reduction in plasma iron; the bone marrow receives little iron for haemoglobin regeneration (bone marrow iron is absent, serum ferritin is <12ug/l, transferrin saturation is <16% and free erythrocyte porphyrin is increased); however, hemoglobin concentration remains normal.
- **Iron deficiency anaemia**: this is a very late stage of iron deficiency with progressive fall in haemoglobin levels and mean corpuscular volume.

Pregnancy outcome in anaemia

In the case of moderate to severe anaemia, breathlessness, oedema, congestive heart failure and even cerebral anoxia have been observed. Two hundred anaemic pregnant women observed in the University Hospital, Institute Medical Sciences, Varanasi, showed a higher incidence of premature
labour and of preterm, low birth weight and stillbirth deliveries. Even the infants born alive had low Apgar scores, and the rate of neonatal deaths was higher. Maternal mortality was 13 in 200 anaemic pregnant women as compared to 1 in 50 controls. Similar findings were reported in other Indian studies. Anaemic mothers do not tolerate blood loss during childbirth; as little as 150 ml can be fatal. Normally, a healthy mother during childbirth may tolerate a blood loss of up to 1000 ml\textsuperscript{1,2}.

**Iron deficiency in India**

As early as 1967, Routh & Agarwal\textsuperscript{3}; studied the iron content in liver, spleen and muscle of healthy rich persons who died in car accidents on the streets of Delhi. It was found that >65% of these healthy persons had nil or very low iron content in hepatic tissue, indicating a severe degree of iron deficiency in our well-to-do population of Delhi. Later, in 1989\textsuperscript{4} national studies by the Indian Council of Medical Research (ICMR)- covering 11 States reported that the prevalence of anaemia (arrived at by estimating haemoglobin using the cyanmethemoglobin method) in pregnant rural women was 87.6%, the mean haemoglobin level being <10.9g/dl. In six of the States, the anaemic women were given various doses of oral iron, 60, 120 and 180 mg, along with 500 µg folic acid daily for 90 days. In the year 1992, 62% continued to be anaemic in spite of receiving iron-folate therapy for 3 months\textsuperscript{5}. This indicates that short-term treatment as recommended in the National Anaemia Control Programme may not be sufficient to control anaemia in pregnancy. However, it was observed that birth weights improved and the incidence of low birth weight deliveries was significantly reduced\textsuperscript{6}. Gomber \textit{et al.}, in 2002\textsuperscript{7}, showed that pregnancy anaemia affects foetal growth. They administered a high dose (335mg) of ferrous sulphate and 500µg of folic acid for 14 weeks as either weekly or daily doses. Both dosing regimens were effective in controlling pregnancy anaemia, thereby suggesting, that the administration of even a once-weekly iron- folate dose can be effective.

The National Family Health Survey 1998-99 (NFHS-2)\textsuperscript{8}, using the hemocue method for estimation, reported the prevalence of anaemia as 49.7% in pregnant women, 56.4% in breastfeeding non-pregnant women, and 50.4% in non-pregnant non-breastfeeding women. The hemocue method over estimates haemoglobin level, and it is therefore difficult to compare these data with those from other national studies. Dr Gopalan raised two questions in relation to the NFHS-2 data

- Why not determine the prevalence and severity of anaemia in the same States/districts and villages covered by the NFHS-2, but this time using the cyanmethemoglobin method? The planned study was for observing the changing trends in anaemia over the years in the light of the earlier data collected using the cyanmethemoglobin method.
- What are the factors responsible for inter-State differences in the prevalence and severity of anaemia?

In 2002-2003, the Nutrition Foundation of India studied the prevalence of anaemia in pregnancy and lactation in 7 States (Assam, Himachal Pradesh,
Haryana, Kerala, Madhya Pradesh, Orissa, and Tamil Nadu). The prevalence of pregnancy anaemia was 86.1% (Hb <7.0 g/dl in 9.5%), and in lactating mothers with infants under 3 months of age, it was 81.7% (Hb <7.0 g/dl in 7.3%). The inter-State differences that were responsible for the differences in the prevalence rates of anaemia were mainly fertility, women's education, nutrition status and occupation, availability of antenatal services and iron folate tablets during pregnancy.

In 1999-2000, the ICMR conducted a District Nutrition Survey in 11 States covering 19 districts. The prevalence of pregnancy anaemia was 84.6% (Hb <7.0 g/dl in 9.9%). These study workers also found that 90% of the adolescent girls in these districts had anaemia. Similarly, in East Delhi schools, 85% of the adolescent girls were iron-deficient and 49% were anaemic. In an ICDS block that has been in operation for more than 20 years in East Delhi, >87% of the under-three age group of children were iron-deficient, and >40% had vitamin B12 deficiency. The above studies clearly show that our populations live with anaemia throughout the entire lifecycle, endangering child growth and development.

Effect of maternal iron deficiency on foeto-placental unit

The high prevalence as well as the severity of anaemia during pregnancy and lactation carry grave connotations. This is the period when a baby’s brain cells grow, neurotransmitters develop, and iron is essential for this process. Normally ‘Placental Iron transfer’ to fetus becomes 3 to 4 times during 20-37 wk of gestation. Cord serum iron and haemoglobin were found to be lower in pre-term as well as full-term infants of hypoferrimic mothers. There is an increased gradient, in the presence of maternal iron deficiency, for transport of iron from mother to foetus, but the transport remains proportional to the degree of maternal hypoferrimia. The placenta plays an important role in maintaining iron transport to the foetus. This process of iron transport is purely a placental function over which mother and foetus have no control; in fact, it has been shown that the placenta continues to “trap” iron even after the foetus is removed in animals. In spite of this efficient protective mechanism, the placental iron content is reduced significantly in maternal hypoferrimia. This was a very important finding, as earlier studies by Vahlquist (1941) and Rios et al. (1975) on Swedish and American women had reported that cord iron does not change in iron-deficient pregnant women.

The placentae of anaemic women showed a qualitative decrease in villous surface area, volume of villi and length of blood vessels, while surface area and volume of the intervillous space increased. These placental changes in anaemia did not normalise after rehabilitation, suggesting that “maturational arrest” had occurred. Foetal liver iron stores are reduced significantly in maternal hypoferrimia. Normally, the bigger the infant and more advanced the gestational age, the higher was the amount of iron in the foetal liver, spleen and kidney. The tissue iron content increases steeply in the last 8 weeks of gestation. Infants born before 36 weeks of gestation had half the iron content in hepatic reserve. The iron content in breast milk is higher in hypoferrimic mothers, a phenomenon of “Physiological Trapping.”
Foetal brain iron content in maternal latent iron deficiency in rats

Iron as a micronutrient is required for the regulation of brain neurotransmitters by altering the enzymatic pathway system. In order to study iron deficiency, a rat model was developed to create iron deficiency (low hepatic iron) without change in haematocrit levels. In postweanling rats, iron decreased irreversibly in all brain parts except medulla oblongata and pons. Susceptibility to iron deficiency showed variable reduction in different parts of the brain: corpus striatum 32%, midbrain 21%, hypothalamus 19%, cerebellum 18%, cerebral cortex 17% and hippocampus 15%. Alterations in brain iron content also induced significant alterations in the percentages of Cu, Zn, Ca, Mn, Pb and Cd.

Foetal latent iron deficiency and brain neurotransmitters in rats

Taneja and Shukla showed that in latent iron deficiency there is irreversible reduction in:

- brain ‘glutamate metabolism’-(GAD, GDH, GABA-T); there was a marked reduction in the levels of brain GABA, L glutamic acid and enzymes for biosynthesis of GABA and L-glutamates like glutamate decarboxylase and glutamate transaminase; the binding of H3Muscimol at pH 7.5 and 1mg protein/assay (GABA receptor) increased by 143%, but glutamate receptor binding decreased in the vesicular membranes of latent iron-deficient rats by 63%.
- brain ‘TCA-cycle’ enzymes; mitochondrial NAD+ linked dehydrogenase reduced significantly
- brain ‘Catecholamine metabolism’; whole-brain-dopamine, neonephrine, tyrosine and TAT reduced significantly; in the corpus striatum the situation was similar, except that TAT increased.
- brain ‘5-HT metabolism’; tryptophan, 5-HT, and 5-HIAA reduced significantly.

The whole-brain and corpus striatum showed reduction in catecholamine, dopamine nor-epinephrine, tyrosine and monoamino oxidase, while tyrosine amino transferase increased in the corpus striatum in spite of reduction in whole-brain, thereby suggesting that latent iron deficiency induces irreversible neurotransmitter alterations. These changes were specific to iron deficiency, because neurotransmitter alterations in the foetal brain on account of malnutrition get normalised partially or completely on rehabilitation. The significant effects on neurotransmitter receptors (glutamate mediators) during the early stages of iron deficiency clearly indicate the deficits in both excitatory and inhibitory pathways of the central nervous system, showing that iron plays an important role in brain development.

To test the above findings in humans, babies born to moderately-to-severely anaemic mothers were examined for “impact of iron deficiency on mental functions”. The intrauterine-growth-retarded offspring of anaemic and undernourished mothers showed:
hypotonia in 72% and hypoxiccitability in 56%
- modification of responses in several neonatal reflexes, e.g. limp posture, poor recoil of limbs, and incomplete Moro’s and crossed extensor responses.
- shortening of sleep cycle (REM and NERM) as evidenced on the EEG, the reduction being more marked for REM sleep. There was some inter and intra-hemispheric asymmetry and abnormal paroxysmal discharges, suggesting dysmaturity of the brain\textsuperscript{31, 32}.

The above findings were not specific to the effects of anaemia on mental functions. Therefore the effects of anaemia (nutrition-controlled) on mental functions were then studied separately in rural children during a period of three years, with the support of the Nutrition Foundation of India.

Mental functions in nutrition-controlled 388 rural primary school children (6-8 yr of age), matched for social and educational status, were studied by WISC and arithmetic test to assess “intelligence, attention and concentration”. Anaemia does not affect intelligence, except subtest-digit span. In arithmetic tests, attention and concentration was poor in anaemic children\textsuperscript{33}.

**Anaemia and brain-MRI studies in humans**

In anaemia caused by iron deficiency (serum ferritin \(<15\mu g/l\)) there is nil or low iron content in body tissues. By contrast, in thalassemia \((>1000\mu g/l)\), there is excess of iron in body tissues. The iron content on globus pallidus, caudate and dentate nuclei was similar in both the clinical conditions, indicating that the deposited iron in brain does not change. In anaemia there was an increase in creatinine and aspartate and reduction in choline concentration. These are significant findings, as choline is synthesized in the brain in very small amounts; its uptake is Na+-dependent, requiring oxygen. Such changes are also observed in Hutzon’s chorea and Alzheimer’s disease\textsuperscript{25}.

**Effects of iron deficiency and/ or anaemia on the brain**

Iron-deficiency anaemia in infancy has been consistently shown to negatively influence performance in psychomotor development. Short-term iron therapy did not improve the lower scores, despite complete haematological replenishment. Neurological maturation was studied in infants 6 months of age, including auditory brain stem responses and nap time 18-lead sleep studies. The central conduction time of the auditory brain stem responses was slower at 6, 12 and 18 months and at 4 years in these children, despite iron therapy having commenced at 6 months. During the sleep-wakefulness cycle, heart rate variability, a developmental expression of the autonomic nervous system, was less mature in anaemic infants. This is possibly due to altered myelination of the auditory nerves\textsuperscript{34}.

Lozoff \textit{et al}\textsuperscript{35}, in their studies on the long-term effects of iron deficiency in infancy; showed that these children, from preschool to adolescence, had poor cognitive, motor, and socio-emotional function, as well as persisting neurophysiological differences (slow transmission of nerve impulses
throughout the brain in auditory and visual systems). This is due to defects in myelination, neurometabolism and neurotransmitter function in iron deficiency. It has been observed that these changes are resistant to iron therapy in children <2 years of age with iron-deficiency anaemia, but not in older children.36 These studies supported our earlier findings that brain functions are significantly affected in latent iron deficiency in the brain growth period, and that such changes are irreversible. These findings have serious consequences, e.g. poor cognition and learning disabilities.

The above research studies by our group are mainly on the effects of latent iron deficiency on irreversible brain function, and neurotransmitter alterations in the brain growth period. Once anaemia sets in, the additional effects are due to anoxia. Our nation is faced with the problem of iron deficiency that leads to anaemia, a clinical condition caused by the deficiency of many nutrients, mainly iron, folic acid and vitamin B12. Folic acid is essential from the prenatal period onwards, and its deficiency causes neural tube defects. In India, the population experiences a whole life cycle of anaemia, endangering child growth and development. Nutritional anaemia is treatable as well as preventable, and the available control measures are affordable. Let’s do it now.

References

5. Indian Council Medical Research. Field supplementation trial in pregnant women with 60, 120 and 180 mg of iron and 500 ug of folic acid ICMR report, New Delhi, 1992.