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Immunobiology of mild micronutrient deficiencies

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Nutrition is a critical determinant of the outcome of host microbe interactions through a modulation of the immune response. Besides macronutrient malnutrition, deficiencies of several macronutrients also influence immune homeostasis and thus affect infection-related morbidity and mortality. Deficiencies of micronutrients like vitamin A, iron and zinc are widely prevalent among populations living in developing countries. Besides their severe deficiencies, subclinical deficiencies are known to impair biological functions in the host, immune function being one of them. The effects of these micronutrients on various immune mechanisms are briefly reviewed in this article.

Zinc: Iron: Vitamin A: Immunity

Nutrition is a critical factor in modulating immune homeostasis and thereby the outcome of host microbe interactions. Micronutrient deficiencies produce a wide spectrum of effects ranging from clinical disorders due to severe deficiency to subtle functional impairment in subclinical deficiencies which could nevertheless, significantly influence the health and survival of the population. It is now well established that besides protein and energy malnutrition, deficiencies of several micronutrients down-regulate immune responsiveness and increase morbidity and mortality due to infections particularly, among children residing in developing countries.

Deficiencies of vitamin A and iron are widely prevalent among preschool children and are important public health problems in most developing countries. Zinc deficiency is believed to be widespread among populations subsisting on cereal based diets due to its poor bioavailability and its effect on the increasing incidence and severity of common childhood infections is becoming apparent.

Vitamin A deficiency

Besides being an important micronutrient modulating vision, vitamin A has several extra ocular functions (Olson, 1986). Its effects on gene expression influence cellular proliferation and differentiation and glycoprotein synthesis, factors that are important determinants of immune function and epithelial cell integrity.

Vitamin A – immune functions

Vitamin A deficiency – innate immune mechanisms

Integrity of the epithelial lining of mucosal surfaces with its mucus covering constitutes the major limb of the innate immune responses and is essential to prevent microbial invasion. Loss of integrity of the epithelial lining of mucus membranes in a vitamin A deficient state explains its close association with increased susceptibility to infections particularly of gastrointestinal, respiratory and genitourinary tracts.

Even mild or subclinical vitamin A deficiency induces keratinizing metaplasia of the epithelium and depletes goblet cells from mucosal linings thus causing xerosis of the membranes (Reddy *et al.* 1989). The xerotic surfaces form potential sites for increased bacterial adherence (Chandra, 1988), thus leading to bacterial colonization.

In the event of systemic invasion by microbes, several humoral and cellular components of the innate immune system get activated and constitute the first order of defence.

The antimicrobial enzyme lysozyme depends on vitamin A for its synthesis. Intracellular lysozyme content has been shown to significantly decline in children suffering from mild xerophthalmia (Mohanram *et al.* 1974).

Neutrophils and macrophages are phagocytic and bactericidal cells. Increase in respiratory burst on contact of a microbe with the plasma membrane of the phagocytes triggers a series of metabolic events resulting in the production of microbicidal molecules such as H_2O_2 and O^{2-}. Studies in experimental vitamin A deficient rats suggest impairment in the phagocytic cell functions (Ongsakul *et al.* 1985). However, studies in subclinically vitamin A deficient children (serum retinol $<0{\cdot}7$ μmol/L) demonstrated no alteration in the bactericidal properties of neutrophils (Bhaskaram *et al.* 1989*a*) and in the IL_1 production and cytotoxic properties of *in vitro* activated macrophages (Bhaskaram *et al.* 1989*b*).

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Vitamin A deficiency and specific immune functions

Besides the effects on the innate immune system, vitamin A also modulates specific immune mechanisms. Impaired development of primary lymphoid organs and impaired cellular proliferation have been demonstrated in vitamin A deficient chicks. These effects were attributed to impairment of lymphoid cellular proliferation and differentiation of primary lymphoid organs and also to changes in the homing patterns of the cells (Takagi & Nakano 1983).

Bhaskaram & Reddy (1975) demonstrated a decrease in T-cell number with no change in proliferative activity in children suffering from mild xerophthalmia due to vitamin A deficiency. Further, decrease in the proportions of T-cell subpopulations have been documented in children having vitamin A deficiency (Bhaskaram, 1989; Semba *et al.* 1993). It is known that vitamin A deficiency affects epithelial cells as clinical lesions become apparent. It is possible that vitamin A deficiency by compromising thymic epithelial integrity might reduce thymulin secretion resulting in these changes. This effect was reversible with adequate vitamin A supplementation. However, mild vitamin A deficiency in children does not appear to have significant effects on humoral immune mechanisms. Antibody titres mounted on challenge with diphtheria and tetanus and typhoid vaccines have been found to be unaltered (Bhaskaram *et al.* 1989*a*). Kutty *et al.* (1981) demonstrated no change in the circulating B-cell number in children with mild vitamin A deficiency.

Vitamin A deficiency – infections

Association of infectious diseases and severe vitamin A deficiency has been well established in both experimental animals (Darip *et al.* 1979) and children with keratomalacia (McLaren *et al.* 1965). These studies demonstrate close association between severe vitamin A deficiency and infection but not necessarily evidence of a causal relationship between the two due to possible confounding influences of other variables particularly, in the human studies. Severe single nutrient deficiencies are rare in humans unlike in experimental situations. Particularly, severe vitamin A deficiency is always associated with severe protein energy malnutrition that also causes immunosuppression. Hence extrapolation of results obtained from experimental studies may not be valid for humans.

However, Sommer *et al.* (1984) reported increase in the relative risk of death in Indonesian children having evidence of mild xerophthalmia. In a subsequent report the same group of investigators reported reversal of these effects following vitamin A administration (Sommer *et al.* 1986). These observations attribute a causal role for vitamin A to increase morbidity and mortality due to infections. This striking observation has triggered a global debate on the role of vitamin A in child survival and led to intervention trials in preschool children in a number of countries under heterogeneous conditions with regard to the vitamin A status, culture, ecology and disease pattern. The results of a meta-analysis of eight major mortality intervention trials (Beaton *et al.* 1993) indicated an overall reduction of mortality rate by 23 % with vitamin A supplementation. Impact of vitamin A was more consistently appreciated with measles (Sommer & West, 1996) and diarrhoea (Barreto *et al.* 1994) rather than on respiratory tract infections in lowering the morbidity and mortality. Though vitamin A deficiency has been associated with acute respiratory infections (Sommer *et al.* 1984), Stephensen *et al.* (1997) reported the impact of vitamin A intervention on respiratory morbidity could be equivocal or even adverse. Herrera *et al.* (1996), however, reported from a large hospital based clinical study that vitamin A had a marginal effect on the severity of the infections, if not on duration. An increase in coughs observed among the supplemented children was however, interpreted as a beneficial response.

The mechanisms involved in these varying outcomes of vitamin A supplements on intestinal and respiratory mucosa are not clearly understood. It is possible that the differences in the milieu to which the mucosal cells of these two systems are exposed and a number of other systemic and environmental factors governing the local tissue response and factors influencing the local mucosal immune response might be the critical determinants of the outcome of intervention.

Irrespective of the mechanism of action, the results of vitamin A supplementation on respiratory morbidity raise concern about vitamin A supplementation programmes implemented in communities with widely prevalent vitamin A deficiency often co-existing with recurrent respiratory infections in children.

Effect of large dose vitamin A

The immune adjuvant functions of vitamin A are well established. Children receiving 100 000 to 200 000 IU of RE as a single oral dose were observed to have enhanced phagocytic functions with increase in hydrogen peroxide production by neutrophils. Macrophage functions measured by interleukin 1 production and cytotoxicity were also enhanced. A significant increase in antibody titres to specific antigens like diphtheria and tetanus and typhoid vaccine was also observed (Bhaskaram *et al.* 1989*a*). Similar observations have also been reported from Bangladesh by Rahman *et al.* (1999). These immune potentiating effects were observed even in healthy children whose serum retinol levels were more than 0·7 μmol/L (Bhaskaram *et al.* 1989*a*).

Co-administration of vitamin A with measles vaccine has been documented to significantly improve seroconversion rates in 9-month-old infants when maternal antibody titres are low or absent (Benn *et al.* 1997). This effect is evident particularly when seroconversion rate to measles vaccine in routine immunization programmes is low as observed in tropical countries like India (Bhaskaram & Rao, 1997). Semba *et al.* (1997) however, found no enhancement of seroconversion rates in 9-month-old Indonesian infants whose basal seroconversion rates were already optimal. However, similar potentiating effects of vitamin A administration were not observed on oral polio vaccine (OPV) immunogenicity when it was administered to young infants (Bhaskaram & Balakrishna, 1998; Bahl *et al.* 1999). It may

be interesting to determine if maternal antibodies in the newborns and young infants explain the lack of an enhancing effect on OPV immunogenicity. Nevertheless, these reports, which clearly indicate no adverse effects of combining administration of vitamin A with live attenuated viral vaccines, are particularly of significance at a time when the safety of such a combined strategy is being questioned.

The immuno-potentiating effects of vitamin A and its related compounds have been exploited in treating immuno-compromised states like burns and surgical conditions (Fusi *et al.* 1984; Cohen *et al.* 1979).

Vitamin A – infection and immunity in pregnancy

Recent studies have highlighted the significant role of vitamin A in pregnant women. Christian *et al.* (1998) from Nepal reported a higher frequency of urinary, reproductive tract and gastrointestinal infections in pregnant women of low vitamin A status. Higher rate of vertical transmission of the disease (Semba *et al.* 1994) and higher rate of vaginal shedding of infected cells (Mostad *et al.* 1997) in pregnant women positive for HIV-1 virus and having low serum retinol levels thus threatening the infant survival have been reported. The investigators speculated that several factors like altered maternal immune function due to the combined effects of vitamin A deficiency and HIV −1 infection in pregnancy with consequent increase in viremia, impaired epithelial integrity of the lower reproductive tract, and a possibly compromised placental function may have been responsible for the high rate of infection of the babies either *in utero* or during labour. Viral excretion in breast milk could be an added risk for breast-fed infants.

Apart from the role of vitamin A deficiency in increasing the morbidity in pregnant women, West *et al.* (1999) in a double blind randomised trial in Nepal, reported that administration of vitamin A or β-carotene supplements led to a 44 % reduction in maternal deaths related to pregnancy and early postpartum period. This study invited substantial criticism and serious concern was expressed particularly regarding the causes of maternal deaths that were prevented by the intervention, as well as the ethical issues concerning the administration of 7000 μg of retinol once a week during pregnancy. These results need confirmation in other situations using appropriate experimental design.

Iron deficiency

Iron deficiency anaemia affects all age groups of the under privileged population in most developing countries. Even among affluent families, specific age groups like adolescent girls and the elderly suffer from this nutrient deficiency. Due to its serious functional effects, iron deficiency anaemia in the vulnerable groups namely pregnant women and preschool children is of public health significance in developing countries. Besides anaemia, subclinical iron deficient status also influences several physiological functions that govern cellular proliferation and metabolism. The significant association of iron deficiency anaemia with infection demonstrated by epidemiological and clinical studies has been extensively reviewed (Bhaskaram, 1988) and the effects attributed to the adverse effects of iron deficiency on the immune system. Reversal of the clinical and immunological defects by iron administration in iron deficient adults suffering from muco-cutaneous candidiasis convincingly demonstrates the casual role for iron in increasing susceptibility to infections (Chandra & Vyas, 1984).

Severe iron deficiency anaemia has been shown to impair cellular immune functions which reverted to normal following correction of the deficiency (Chandra & Saraya, 1975; MacDougall *et al.* 1978). In a subsequent study by Srikantia *et al.* (1976), Indian children suffering from moderate to severe anaemia (<100 g/L) were found to have impaired neutrophil bactericidal activities and cell mediated immune functions which were reversible with adequate iron therapy (Bhaskaram *et al.* 1977). Prasad (1980) demonstrated a decrease in halogenation of phagocytosed particles in neutrophils in iron deficient individuals, a function that is carried out by the iron dependent myeloperoxidase enzyme which constitutes a powerful bactericidal system in neutrophils in the presence of H_2O_2 and halide ions. Impaired cell mediated immune functions have also been demonstrated in anaemic pregnant women (Prema *et al.* 1982). Sipahi *et al.* (1998) observed circulating IL_2 levels to be low in children suffering from iron deficiency anaemia. In confirmation with the previous study, recent studies from the National Institute of Nutrition, Hyderabad, India (unpublished) demonstrated polarization of T-lymphocyte subsets to Th_2 population with predominant IL_4 mRNA expression with no IL_2 expression in anaemic children unlike the normals in whom both IL_2 and IL_4 were expressed. This polarization can interfere with resistance to infections particularly due to intracellular pathogens and also response to certain vaccines. The irreversible effect of maternal anaemia on the immune functions of the offspring observed in the experimental animals (Kochanowski & Sherman, 1983) is a further cause for concern in populations with high prevalence of iron deficiency in pregnancy.

Zinc deficiency

The extent of zinc deficiency has not been precisely quantified among populations because of lack of a simple and sensitive tool to measure zinc status. Nevertheless, the low bioavailability of zinc from cereal-based staple diets of populations in most of the developing countries has led to the presumption that mild or subclinical zinc deficiency could be widespread in such populations.

Zinc – infectious diseases

The close association between zinc deficiency and increased diarrhoeal and respiratory morbidity has been demonstrated (Bhandari *et al.* 1996; Bahl *et al.* 1998). Several investigators have demonstrated a significant and consistent reduction in the severity and duration of diarrhoea following zinc administration particularly, when it is prolonged (Roy *et al.* 1999; Penny *et al.* 1999; Sazawal *et al.* 1997*a*; Sazawal *et al.* 1995) by supplementing with

zinc in varying doses. Further, Ruel *et al.* (1997) from Guatemala documented significant reduction of acute respiratory infections following zinc supplementation in children. Sempertegui *et al.* (1996) documented the usefulness of zinc supplements to reduce respiratory tract infections in malnourished children. These beneficial effects observed with zinc supplements on both diarrhoeal and respiratory morbidity have practical significance and suggest that zinc supplements may complement the effects of vitamin A in reducing over all morbidity burden due to infections in populations having both these micronutrient deficiencies. However, there is a need to establish the safety of zinc supplementation in communities where other trace metal deficiencies are equally widespread as prolonged zinc administration is known to interfere with the absorption of copper, iron and calcium.

Zinc supplements were also found to be beneficial in the elderly (Mocchegiani *et al.* 1999) in reducing opportunistic infections by preventing reduction in CD_4^+ cells. Due to similar immune effects, it has also been demonstrated to be useful as an adjunct to chemotherapy in individuals suffering from acquired immune deficiency syndrome (AIDS) (Mocchegiani *et al.* 1995). The effects of zinc on infectious diseases may be attributed to its effects on varied immune functions. However, it is not clear whether these actions of zinc on infections and immune functions are brought about by correcting the existing zinc deficiency or by pharmacological actions.

Zinc and immune function

Zinc is essential for DNA synthesis and is a co-factor for several enzymes including thymulin which regulates the intrathymic differentiation and maturation of T-lymphocytes. Beck *et al.* (1997) observed decrease in Th_1 cytokines with no change in Th_2 cytokines thus causing an imbalance between Th_1 and Th_2 responses in experimentally induced zinc deficiency in humans. Zinc was shown to expand the IL_2 (interleukin 2) producing lymphocyte population in *in vitro* studies (Saha *et al.* 1995). Sazawal *et al.* (1997*b*) reported a significant increase in CD_3, CD_4 lymphocyte populations with an increase in CD_4: CD_8 ratio following zinc supplementation in children.

Besides these effects of zinc on cell mediated immune responses, its role in modulating phagocyte and NK cell activity are well described. Fan *et al.* (1996) observed that impaired leucocyte adhesion and lymphocyte function in zinc deficient premature infants were reversed following zinc therapy. Abul *et al.* (1995) documented the regulatory role of zinc on the production of IL-1 α from alveolar macrophages of patients with acute lung disease. In addition to these effects, zinc supplements lead to accelerated regeneration of mucosa, increase in the levels of brush border enzymes, enhanced cellular immunity and higher level of secretory antibodies as documented by Folwaczny *et al.* (1997). These varied and protective immunological functions of zinc perhaps explain its effects in significantly reducing infectious illness particularly those of mucosal tracts.

Antioxidant micronutrient deficiency

A number of micronutrients like β carotene, vitamin C, selenium, copper and others are powerful antioxidants and have a significant impact on infection related morbidity in humans. Oxidant stress has been demonstrated to have impact on viral infections. By influencing the cytokine profile of T-cells and also by inducing genomic alterations in the viruses, oxidant stress plays a role in enhancing the pathogenesis of a virus or leads to the emergence of new pathogenic viral strains. Deficiency of dietary antioxidants increases this risk (Beck & Levander, 1998). This could be a potentially serious threat particularly, in the micronutrient deficient developing countries.

Conclusions

Micronutrient deficiencies are of clinical and public health magnitude in developing countries and account for significant morbidity and mortality due to infections. Vitamin A, zinc and iron, the micronutrients of clinical and public health significance have immunomodulatory effects by influencing a wide range of immune mechanisms in humans. Though the effects of single micronutrients are well established in experimental animals, such isolated deficiencies are uncommon in humans. The complex interactions of several coexisting nutritional deficiencies particularly of trace metals warrant studies to establish the bioeffects and cost effectiveness of providing single nutrient supplements vis-à-vis multinutrient supplements. Defining the precise extent of the problem contributed by deficiency of each micronutrient is essential though challenging. The untoward effects that follow prolonged single nutrient supplements (e.g. prolonged zinc supplements on the nutrients like copper, calcium and iron) need to be explored and the ethical issues concerning single nutrient supplements to populations having multiple deficiencies critically addressed.

Though this article highlights only the immunobiology of some commonly deficient micronutrients, it is needless to mention that several other micro and macronutrient deficiencies could be concomitantly present in the underprivileged populations with several other functional deficits which need correction together to achieve significant effects on the overall health of the population rather than promoting mere survival.

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