SERUM BETA-CAROTENE DEFICIENCY IN HIV-INFECTED CHILDREN

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Representative levels of serum micronutrients specifically, beta-carotene and vitamins A and E, were studied in symptomatic human immunodeficiency virus (HIV)-infected children. The nutritional status of 23 symptomatic African-American and Hispanic HIV-infected children were compared with an appropriate control group comprised of 36 uninfected children matched for age and sex, using body mass index. Serum beta-carotene and vitamin A and E levels were randomly determined on 15 of the infected children.

Beta-carotene concentration was 4.9-fold reduced in symptomatic HIV-infected children when compared with the control group. There was a 6.5-fold decrease in the serum level for children without acquired immunodeficiency syndrome (AIDS) and a 13-fold reduction in children with AIDS. No differences in the mean values for serum vitamins A and E were observed in the groups studied. Although the nutritional status of the symptomatic HIV-infected children was not different from that of the control population, their serum beta-carotene levels were profoundly deficient. This finding may have immunologic and clinical implications for children with rapidly progressing HIV disease. (*J Natl Med Assoc.* 1996;88:789-793.)

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The progression of disease in children infected with human immunodeficiency virus (HIV) may be rapid. The reasons for the rapid deterioration of the immunological status in some infants while others remain relatively stable have not been adequately studied. Malnutrition has been implicated as a contributing factor to immune dysfunction, specifically abnormalities of B and T cells, by enhancing the susceptibility to HIV infection and progression of the disease.[1,2] Failure to thrive, a manifestation of malnutrition, is a cardinal feature of pediatric HIV infection. The extent of macro- and micronutrient deficiencies in HIV-infected children is not well-defined.

Vitamins A and E, beta-carotene, and glutathione have been studied to a large extent in adult patients with HIV infection.[3,4] Bull et al[5] demonstrated systemic glutathione deficiency in asymptomatic HIV infected individuals, while Staal et al[6] stressed that the latency period of HIV infection may be extended by the levels of glutathione. The protective effects of glutathione derive partly from its ability to remove hydrogen peroxide from the cells while vitamins A and E and beta-carotene, on the other hand, scavenge free radicals directly. Specifically, beta-carotene protects the lipid components of tissue from oxidation.[7-9] Furthermore, beta-carotene has immunostimulatory effects on both HIV-infected patients as well as healthy humans.[10,11]

Apart from its stimulatory effect on the immune system and the maintenance of epithelial integrity,[12] vitamin A supplementation reduces childhood mortality and morbidity in nutritionally deprived populations.[13,14]

TABLE 1. COMPARISON OF SERUM BETA-CAROTENE AND VITAMIN A AND E LEVELS, AGE, CD4 COUNTS, AND BODY MASS INDEX IN CONTROLS AND SYMPTOMATIC PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS

Parameter*	Controls	No. Subjects	Symptomatic	No. Subjects	*P* Value
Age (years)	4.4 (4.9)	38	4.6 (3.6)	24	>.1
Body mass index	19.8 (2.0)	36	16.0 (4.1)	23	>.1
CD4 (cells/mm^3)	2324 (1331)	31	576 (566)	23	.000005
Serum beta-carotene (μmol/L)	0.39 (0.39)	29	0.08 (0.1)	15	.006
Serum vitamin A (μmol/L)	3.66 (0.91)	14	3.52 (1.18)	12	>.1
Serum vitamin E (μmol/L)	52.7 (16)	14	44.3 (23)	12	>.1

*All values are reported as means, with standard deviations indicated in parenthesis.

Semba et al[15] reported increased mortality in HIV-infected adults deficient in vitamin A. Studies conducted in Rwanda indicate that a deficiency in vitamin A in HIV-infected mothers is associated with high perinatal and infant mortality.[16] Coutsoudis et al,[17] in a recent study, showed that vitamin A supplementation for South African HIV-infected children with normal vitamin A concentration reduced the episodes of diarrhea. Vitamin A-deficient mothers[18] have a fourfold increased risk for perinatal transmission of HIV from mother to child. Furthermore, decreased circulating CD4 naive T cells were reported in malnourished children with xerophthalmia.[19] We therefore were prompted to determine the levels of serum vitamins A and E and beta-carotene in symptomatic HIV-infected children to define the extent of deficiencies in this patient population.

MATERIALS AND METHODS

Twenty-four symptomatic HIV-infected children attending the Pediatric-Maternal HIV Clinic at Woodhull Medical and Mental Health Center, Brooklyn, New York, were studied. Human immunodeficiency virus infection in children was defined as infants and children with positive HIV serology by enzyme-linked immunoabsorbent assay (ELISA) and confirmed by Western blot analysis after 18 months of age. In children younger than 18 months, the diagnosis was made if there was a positive HIV culture or positive polymerase chain reaction (PCR) and clinical signs and symptoms consistent with the diagnosis of HIV infection. The laboratory studies that included HIV serology, PCR, HIV-culture, p24 antigen, immunoglobulins, and T-lymphocyte subsets were performed at the Wardsworth Center for Laboratories and Research, the Department of Health, Albany, New York. Control subjects were either HIV-positive children who had seroreverted or HIV-seronegative siblings of symptomatic infected children. Anthropometric data were collected at every clinic visit when CD4-lymphocyte counts were obtained.

Excluded from the study were patients with chronic diarrhea defined as more than three loose bowel movements per day for at least 1 week. Also, patients with significant hepatic dysfunction as determined by serum bilirubin (>17 umol/L) and aspartate aminotransferase higher than three times the upper limit of normal were excluded. None of the patients had fever at the time of study. Medical, nutritional, and social history were obtained, and a physical examination also was performed and recorded on all children studied. Nutritional status was assessed using body mass index (BMI), which is determined by the formula weightt (kg)/h^2 (m).

The study was approved by the hospital's human research committee, and informed consent was obtained from each parent.

Vitamin Assays

Blood samples were randomly obtained from 15 of the 24 symptomatic HIV-infected children. The blood was allowed to clot for about 45 minutes before it was centrifuged; the serum was separated and stored frozen in a clean test tube at −73°C until assayed for vitamins A and E and beta-carotene, using reversed phase high-pressure liquid chromatography as described elsewhere.[20-22]

Statistical Analysis

Statistical analysis was performed with the aid of the Number Cruncher Statistical system software program (Version 5; Kaysville, Utah). Group comparisons were made using the Mann-Whitney two-sample test.

RESULTS

Table 1 shows the mean age, BMI, CD4-lymphocyte count, serum beta-carotene, and vitamin A and E levels for the symptomatic HIV-infected children and the control group. Of the 15 HIV symptomatic children on whom vitamin levels were determined, 8 had full-blown acquired immunodeficiency syndrome (AIDS) and 7 were without AIDS (Table 2). The Centers for Disease Control and Prevention's (CDC) classification system for HIV in children was used to identify AIDS and non-AIDS patients.[23] The mean CD4 lymphocyte count was significantly decreased in symptomatic children with HIV infection. There was a modest correlation (r=0.44), although not statistically significant, between CD4 lymphocyte counts and serum beta-carotene levels in symptomatic HIV-infected subjects. The beta-carotene concentration was 4.9-fold reduced in symptomatic children.

Symptomatic HIV-infected children were further stratified into two groups: children with AIDS and non-AIDS (Table 2). Beta-carotene level was 13-fold decreased in AIDS patients when compared with controls. Although the BMI for AIDS and non-AIDS patients were not statistically different, there was a 50% reduction of beta-carotene concentration in patients with AIDS. Similarly, the CD4 lymphocyte count also was reduced by 50% in children classified as having AIDS.

TABLE 2. COMPARISON OF BETA-CAROTENE LEVEL AND CD4-LYMPHOCYTE COUNT FOR CHILDREN WITH AND WITHOUT ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)*

	Non-AIDS	AIDS
Stage of disease	P_2 A (n†=7) P_2 D (n=2)	P_2 C (n=6);
Age (years)	5.57 (5.52)	5.73 (5.73)
Body mass index (kg/h^2)	17.04 (1.23)	15.70 (1.14)
CD4 lymphocytes	743 (608)	369 (406)
Beta-carotene (μmol/L)	0.06 (0.07)	0.03 (0.04)

*All values are reported as means, with standard deviations indicated in parentheses.
†n=number of subjects in each group.

DISCUSSION

The significant finding in the present study was a 4.9-fold reduction (P=.006) in serum beta-carotene concentration in HIV-infected children. Furthermore, patients with AIDS have a 50% reduction of beta-carotene levels when compared with non-AIDS patients (Table 2). The levels of vitamins A and E were not statistically different in the groups studied. Although previous investigators[24-26] have reported normal serum values for vitamin A in HIV infection, as was also demonstrated in the present study, single retinol determinations generally are regarded as insensitive indicators of vitamin A deficiency.[12] Wood and coworkers,[12] using conjunctival impression cytology (CIC) as an end-organ response to available vitamin A, showed that 32% of their patients were deficient in vitamin A despite normal serum concentration in 92% of the cases. Therefore, we cannot conclude from the present study that our HIV-infected children had adequate amounts of vitamin A stores. This is more so, since a recent study from South Africa[17] showed that vitamin A supplementation for HIV-infected children with normal measurable vitamin A levels reduced morbidity associated with diarrhea.

To our knowledge, this is the first study documenting reduced concentrations of serum beta-carotene in HIV-infected children. The decrease is more profound in children with AIDS. The reason for the observed decrease is not entirely clear. There are, however, several plausible explanations. Serum carotene concentration is known to be deficient in HIV-infected persons with or without malnutrition.[4,24] However, the mean BMI for the infected children was not different from that of the control group whose dietary quality and intake were similar to those of the study subjects. Although the dietary intake of beta-carotene was not specifically determined in our patients, carotenoid consumption below the recommended daily allowance is a rare phenomenon in developed countries.[25] It therefore is unlikely that reduced intake was responsible for the low concentration of beta-carotene observed in our study population (Table 2).

The most likely mechanism for the beta-carotene deficiency may be related to the pathophysiological consequences of HIV infection per se. Antioxidants achieve their immunomodulating effects through several mechanisms; for instance, they can block the activation of nuclear factor-Kappa B (NF-kB), which promotes HIV replication via a mechanism using free radicals.[26,27] Beta-carotene is a known potent quencher of free radicals,[7] and its deficiency may impair the elimination of free radicals generated, a situation that can enhance the activation of NF-kB. Another mechanism could involve tumor necrosis factor-alpha, a powerful inducer of apoptosis,[28] which is mediated by oxidative stress secondary to chronic macrophage activation.[27] Oxidative stress therefore may play a role in the pathogenesis of CD4-lymphocyte depletion if antioxidant levels are reduced.

A third pathway involves cellular protection by beta-carotene against lipid peroxidation. In this regard, Allard et al[9] recently demonstrated reduced lipid peroxidation in healthy smokers who received a daily supplement of beta-carotene. It was suggested that the protective effect of beta-carotene on lipid peroxidation derives from its ability to react directly with peroxyl radicals involved in lipid peroxidation.[8,29,30] It is therefore conceivable that a deficiency of beta-carotene may cause uninhibited cellular membrane peroxidation and cellular damage, including damage to CD4-lymphocytes. This reasoning is in accord with the recent finding by Murate et al[11] who concluded that changes of lymphocyte subsets in HIV infection are related to beta-carotene levels. Consequently, beta-carotene deficiency, along with other antioxidant deficiencies, may contribute indirectly to the immunological deterioration seen in HIV infection by facilitating CD4-lymphocyte apoptosis, promoting the activation of the triggers of HIV gene expression such as NF-kB, and by enhancing lipid peroxidation.

CONCLUSION

Symptomatic HIV-infected children in this study, particularly those children with AIDS, had profound deficiencies in serum beta-carotene concentration. Further studies are needed to better define the biological consequences of and the mechanism for the observed deficiency. The possible beneficial effects of vitamin supplementation on immune response and the clinical implications for infants and children with rapidly progressing HIV disease are questions worthy of further investigation.

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