

Original Article

Vitamin A deficiency in patients with diarrhea and HIV infection in Ethiopia

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Diarrhea, micronutrient deficiencies and HIV/AIDS are major public health problems in developing countries, especially in sub-Saharan Africa. This study was aimed to investigate serum levels of vitamin A in diarrheic patients with and without HIV co-infection compared to healthy controls. Two hundred eleven diarrheic patients (110 HIV infected), 87 apparently healthy controls and 41 asymptomatic HIV seropositive blood donors who visited the University of Gondar Hospital, in Gondar, Ethiopia were included. Stool samples were examined for enteropathogens following the standard procedures. Serum vitamin A levels were measured by high performance liquid chromatography. *Shigella* species were isolated from 8.5% of the patients while intestinal parasites were detected in 32.2% without significant difference by HIV serostatus. The mean±SD serum vitamin A in diarrheic patients with (0.82±0.59 µmol/L) and without (0.84±0.54 µmol/L) HIV co-infection and in asymptomatic HIV infected blood donors (0.96±0.52 µmol/L) was significantly lower than that in healthy controls (1.52±0.71 µmol/L), $p < 0.001$. Vitamin A deficiency (VAD, serum retinol < 0.70 µmol/L) was observed in 52.7% and 45.5% of diarrheic patients with and without HIV co-infection, respectively. About 13% of healthy controls and 29.3% of asymptomatic HIV infected blood donors were deficient in vitamin A. The levels of serum vitamin A were not associated with the presence of intestinal parasites or *Shigella* species. The findings demonstrate that VAD is a severe public health problem among diarrheic patients in Gondar, Ethiopia. Intervention programmes involving health and nutrition education and supplementation of vitamin A might help in reducing morbidity in such patients.

Key Words: vitamin A deficiency, diarrhea, HIV, Gondar, Ethiopia

Introduction

Diarrheal diseases are one of the most important causes of morbidity and mortality in developing countries.¹ The situation is severe in sub-Saharan Africa, a region where an estimated 25.8 million adults and children are infected with HIV.² Diarrhea, the passage of loose or watery stools at least three times in 24 hours, is one of the clinical manifestations of HIV infection and usually tends to be chronic.³ Chronic diarrhea, an episode that begins acutely and lasts for more than four weeks,³ in tropical countries is associated with weight loss and is often the presenting illness of HIV infected individuals. This diarrhea wasting syndrome in association with a positive HIV serology test is an AIDS defining illness in the World Health Organization's classification.⁴

On the other hand, micronutrient malnutrition is a major public-health problem in developing countries.⁵ Amongst the many micronutrients, the link between vitamin A deficiency (VAD) and morbidity and mortality from infectious diseases has been known for several years.⁶ The vitamin is an important micronutrient which aids in modulating nor-

mal immune function through lymphopoiesis and cellular differentiation.⁷ VAD may lead to alterations in immunity, including pathological changes in the mucosal surfaces, impaired antibody responses to challenge protein antigens, decreased CD4+ cell populations, and altered T and B-cell functions.⁷ In HIV infected individuals, VAD is associated with the development of HIV related disease sequelae and increased progression to AIDS and death.^{8,9}

In Ethiopia, as in other sub-Saharan Africa, morbidities from diarrhoeal diseases¹ and HIV/AIDS^{2,10} are serious health problems. However, studies assessing the interactions between diarrheal diseases, HIV/AIDS and micronutrient status are non-existent.

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Therefore, the present study was undertaken to investigate the status and magnitude of vitamin A deficiency among diarrheic patients with and without HIV co-infection in comparison to that in healthy controls and asymptomatic HIV infected subjects.

Study population and methods

Study design and subjects

In this cross-sectional study, consecutive diarrheic patients diagnosed at the outpatient department of the University of Gondar Hospital, in Gondar, Ethiopia from March to October 2003 were included. Apparently healthy individuals who had visited the University Hospital as blood donors in the same period like the cases were included as controls. All the controls were residing in the same region with the patients. Blood donors with malnutrition, acute or chronic diseases, as required by the blood banking unit of the hospital, were not included. Informed consent was obtained from all subjects and the study was approved by the Research Ethics Committee of the University.

Collection of stool specimens and examination for enteropathogens

Diarrheic stool specimens were collected in sterile containers and processed immediately following the standard procedures. The specimens were inoculated on plates of MacConkey agar (DIFCO) and Salmonella-Shigella agar (DIFCO). The plates were incubated at 37°C for 24 hours and then examined for non-lactose fermenting colonies which were further examined by conventional biochemical tests for the identification of *Shigella* species and *Salmonella* species.¹¹ The stool samples were also examined microscopically for intestinal parasites following direct, concentration and modified acid fast staining procedures.¹²

Blood collection, serum separation and HIV serology

Blood samples were collected from the patients and controls. Sera were separated by centrifugation and stored at -20°C until used. The presence of HIV antibodies was determined by an enzyme linked immunosorbent assay following the manufacturer's instruction (Vironostica HIV Uni-Form II plus O, Organon Teknika, Boxtel, the Netherlands).

Determination of vitamin A

Serum vitamin A level was determined using high performance liquid chromatography (HPLC) according to the method of Arroyave *et al.*¹³ Serum was deproteinised with an alcoholic solution of retinyl acetate and vitamin A was extracted into hexane. The organic layer was separated, evaporated to dryness under a steam of nitrogen, reconstituted in methanol and injected into HPLC. A Shiseido HPLC system (Shiseido Co. Ltd., Tokyo, Japan) which consisted of separation of retinol by a reverse phase column (Capcell Pak C18 MG S-5, 3x250 mm, 5 µm, Shiseido, Japan) and ultraviolet detection at 325 nm with column temperature set at 40°C was used. Methanol was used as a mobile phase with flow rate adjusted at 500 µl/min. All extraction procedures were carried out under reduced light in order to prevent oxidation of the com-

ponent. Pooled human sera were used to measure intra and inter-assay coefficients of variation in laboratory analyses for serum retinol and found to be 6.5% and 3.3%, respectively.

Statistical analysis

Data were analyzed using SPSS version 10 statistical package (SPSS, Inc., Chicago, IL, USA). A one-sample Kolmogorov-Smirnov test was used to assess whether the data were normally distributed. Serum vitamin A values were log transformed for analysis. The significance of differences in the serum vitamin A levels amongst diarrheic patients with HIV co-infection, diarrheic patients without HIV co-infection, asymptomatic HIV infected blood donors and healthy controls was evaluated using a one way analysis of variance with post hoc Tukey test to determine pairs of means which differ significantly. VAD was defined as serum retinol level below 0.70 µmol/L.¹⁴ Logistic regression was used to find the relation between VAD and baseline parameters of the subjects. A *p* value of < 0.05 was considered statistically significant.

Results

A total of 211 diarrheic patients (110 HIV seropositive and 101 HIV seronegative), 87 apparently healthy controls and 41 asymptomatic HIV seropositive blood donors were included in the study. Table 1 shows baseline characteristics of the diarrheic patients in association with the proportion of patients with VAD by HIV serostatus. Acute and chronic diarrhea was observed in 52.1% and 47.9% of the patients, respectively. The proportion of patients with chronic diarrhea and VAD was significantly higher in those with HIV co-infection (*p* < 0.05).

The mean±SD serum vitamin A levels were not significantly different between diarrheic patients with (0.82±0.59 µmol/L) and without (0.84±0.54 µmol/L) HIV co-infection. However, its levels in the sera of diarrheic patients and asymptomatic HIV seropositive blood donors (0.96±0.52 µmol/L) were significantly lower compared to that in healthy controls (1.52±0.71 µmol/L) (*p* < 0.001). Deficiency of vitamin A was seen in 52.7% and 45.5% of diarrheic patients with and without HIV co-infection, respectively. About 13% of healthy controls and 29.3% of asymptomatic HIV infected blood donors were deficient in vitamin A (Table 2).

Figure 1 shows frequency distribution of serum vitamin A levels in diarrheic patients, asymptomatic HIV infected blood donors and healthy controls. The proportion of diarrheic patients with serum retinol levels consistent with moderate (0.35-0.69 µmol/L) and severe (0.00-0.34 µmol/L) VAD was significantly higher than that in healthy controls and asymptomatic HIV infected blood donors (*p* < 0.05).

Shigella species and intestinal parasites were detected in 8.5% and 32.2% of the patients, respectively. While 82.4% of those with intestinal parasites were infected with single species, infection by two parasites was seen in 17.8%. Intestinal parasites detected in the stools of the patients were *Entamoeba histolytica* (10.0%), *Ascaris lumbricoides* (6.2%), *Giardia lamblia* (5.7%), *Strongyloides stercoralis* (5.7%), *Schistosoma mansoni* (4.3%), hookworm species (4.3%), and *Cryptosporidium parvum*

Table 1. Baseline characteristics of diarrheic patients in relation to vitamin A deficiency

Characteristics	Diarrheic patients with HIV co-infection		Diarrheic patients without HIV co-infection	
	No. of patients	No. (%) with VAD	No. of patients	No. (%) with VAD
Sex				
Male	66	29 (43.9)	65	30 (46.2)
Female	44	29 (65.9)	36	16 (44.4)
Age in years				
15 - 24	11	7 (63.6)	41	17 (41.5)
25 - 34	55	33 (60.0)	31	16 (51.6)
35 - 44	33	12 (36.4)	14	9 (64.3)
>45	11	6 (54.5)	15	4 (26.7)
Residence				
Urban	77	40 (51.9)	64	29 (45.3)
Rural	33	18 (54.5)	37	18 (48.6)
Marital status				
Married	54	26 (48.1)	50	28 (56.0)
Single	33	18 (54.5)	45	17 (37.8)
Divorced	23	14 (60.9)	6	1 (16.7)
Occupation				
Government employee	26	12 (46.2)	22	7 (31.8)
Farmer	46	24 (52.2)	17	11 (64.7)
Student	17	10 (58.8)	10	7 (70.0)
Housewife	7	4 (57.1)	32	13 (40.6)
Other	14	8 (57.1)	20	8 (40.00)
Income*				
Low	59	32 (54.2)	70	34 (48.6)
Medium	34	16 (47.1)	20	8 (40.0)
High	17	10 (58.8)	11	4 (36.4)
Diarrhoea				
Acute	49	20 (40.8)	61	29 (47.5)
Chronic	61	38 (62.3)	40	17 (42.5)
<i>Shigella</i> species				
Positive	4	4 (100.0)	14	7 (50.0)
Negative	106	54 (50.9)	91	39 (44.8)
Intestinal parasites				
Positive	30	17 (56.7)	38	19 (50.0)
Negative	80	41 (51.3)	63	27 (42.9)

* Low: less than 1000 Ethiopian Birr/month. Medium: 1000 – 5000 Ethiopian Birr, High: more than 5000 Ethiopian Birr/month. 1 Ethiopian Birr = 0.12 US Dollar

Table 2. Serum levels of vitamin A in diarrheic patients with and without HIV co-infection, apparently healthy controls and asymptomatic HIV infected blood donors

Vitamin A (µmol/L)	Control (n=87)	HIV seropositive (n=41)	Patients with diarrhea (n=101)	Patients with diarrhea & HIV (n=110)
Mean±SD	1.52±0.71	0.96±0.52*	0.84±0.54*	0.82±0.59*
Median (Range)	1.44 (0.25-4.60)	0.85 (0.10-2.08)	0.85 (0.12-2.87)	0.65 (0.02-3.16)
<0.70, no. (%)	11 (12.6)	12 (29.3)	46 (45.5)	58 (52.7)

* $p < 0.05$ compared to controls.

(1.9%). There was no significant difference in the levels of serum vitamin A in diarrheic patients with and without shigellosis or with and without intestinal parasitoses (Table 3). The serum vitamin A levels in the patients who were found positive for *Shigella* species or intestinal parasites was not significantly different by the presence or absence of HIV co-infection (Table 3).

Regression analyses of age, sex, residence, marital status, occupation, monthly income and the status of enteropathogens as independent variables and serum level of vitamin A as dependent variable did not show any significant association between the parameters and a deficient level of serum vitamin A.

Discussion

HIV infection has become the dominant health problem in many parts of sub-Saharan Africa, with the worst affected areas in central, south and eastern parts of the subcontinent including Ethiopia.² One of the major manifestations of the HIV disease in the region is the diarrhea-wasting syndrome.^{15,16} Patients with wasting syndrome were reported to have low levels of plasma micronutrients, including vitamin A, compared to that in non-wasting patients.^{16,17}

In the present study, 52.7% of the diarrheic patients co-infected with HIV, 45.5% of patients without HIV co-infection and 29.3% of asymptomatic HIV infected

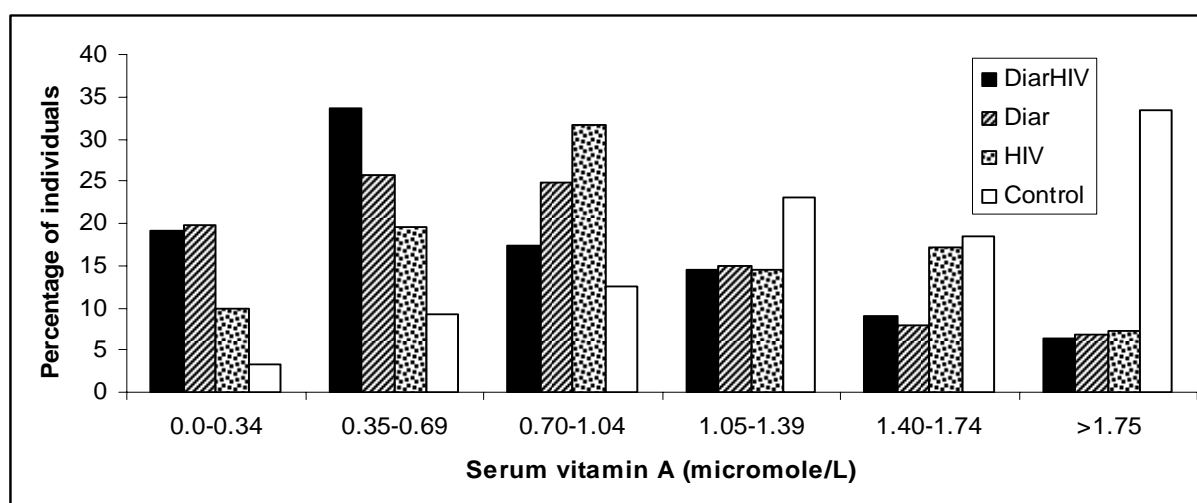


Figure 1. Frequency distribution of serum vitamin A concentrations in diarrheic patients with or without HIV co-infection in comparison to apparently healthy controls and asymptomatic HIV infected blood donors in Gondar, Ethiopia. DiarHIV: patients with diarrhea and HIV co-infection

Table 3. Serum levels of vitamin A ($\mu\text{mol/L}$) in diarrheic patients with and without HIV co-infection by status of enteropathogens

Variables	HIV seronegative	HIV seropositive	Total	<i>p</i> -value
Positive for <i>Shigella</i> species				
Number of patients	14	4	18	
Mean \pm SD	0.85 \pm 0.61	0.39 \pm 0.25	0.75 \pm 0.58	0.2
Range	0.16-2.16	0.13-0.68	0.13-2.16	
Negative for <i>Shigella</i> species				
Number of patients	87	106	193	
Mean \pm SD	0.84 \pm 0.54	0.84 \pm 0.59	0.84 \pm 0.57	0.9
Range	0.12-2.87	0.02-3.16	0.02-3.16	
Positive for intestinal parasites				
Number of patients	38	30	68	
Mean \pm SD	0.74 \pm 0.47	0.81 \pm 0.64	0.77 \pm 0.55	0.6
Range	0.12-1.86	0.20-3.16	0.12-3.16	
Negative for intestinal parasites				
Number of patients	80	63	143	
Mean \pm SD	0.83 \pm 0.57	0.91 \pm 0.58	0.86 \pm 0.57	0.4
Range	0.02-2.77	0.14-2.87	0.02-2.87	

subjects had serum retinol levels below 0.70 $\mu\text{mol/L}$. This observation is in agreement with a few previous reports in African patients.^{16,18-21} Plasma vitamin A levels consistent with deficiency have been reported to occur in 63% of Zambian patients with persistent diarrhea,¹⁶ in 50-63% of HIV infected South African pregnant women,¹⁹ in 29% of Rwandan tuberculosis patients co-infected with HIV,²⁰ in 90% of Tanzanian tuberculosis patients with and without HIV co-infection.²¹ Low vitamin A levels were associated with increased risk of mortality in AIDS patients with diarrhea in Zambia.¹⁶ Despite the reported effect of HIV infection on serum vitamin A status,^{8,9,19-21} we did not observe a significant difference in the mean serum retinol concentrations between diarrheic patients with and without HIV co-infection. This observation may suggest that the ongoing diarrheal illness has a greater impact on retinol status than the effect of HIV infection per se, although further studies are needed to fully understand the discrepancy.

The low serum level of vitamin A in the sera of the diarrheic patients can be due to reduced dietary intake resulting from anorexia or decreased absorption. Further-

more, acute infectious diarrhea is associated with fever which in turn may compromise vitamin A status by increasing urinary excretion.^{22,23} Significant losses of retinol and retinol binding proteins in urine, in a phenomenon known as febrile proteinuria which describes defects in protein reabsorption by proximal tubular epithelial cells of the renal glomeruli and mediated by inflammatory cytokines, has been reported in adult patients with sepsis and pneumonia²² and in children with febrile diarrhea.²³ Low serum retinol levels can also result from impaired ability of the liver to mobilize vitamin A due to the inflammatory response initiated by infection thereby contributing to tissue depletion of retinoids needed for the repair of infection damaged epithelia.²⁴

Our observation that about 13% of HIV seronegative controls and 29.3% of asymptomatic HIV seropositive blood donors had low serum vitamin A levels indicates that VAD is a public health problem in adult population of the region. In line with this, a review of numerous previous studies involving children in Ethiopia indicated the severity of VAD.²⁵ Further, the occurrence of VAD during diarrhea and HIV infection may have clinical

importance because vitamin A is an essential micronutrient for normal immune function.²⁶ During HIV infection, VAD has been associated with increased morbidity and mortality,^{9,27} immune abnormalities,^{26,28} high mother-to-child transmission of HIV,²⁹ higher infant mortality,²⁷ and increased breast milk viral burden.³⁰ Furthermore, laboratory studies suggest that vitamin A deficiency results in impaired antibody mediated immunity and barrier defenses which play great role on protection against infections.²⁶ The synergistic effect of poor nutritional status and diarrhea on immunity to infection has been well described.³¹ Poor nutrition adversely affects an individual's ability to resist or respond to infection, and infection adversely affects the individual's appetite and ability to effectively use energy and nutrients obtained from the diet. The extent to which immunity is impaired is likely to vary with seasonal nutrient intake and distribution of infectious diseases.³¹

Shigellosis remains one of the most severe enteric infections affecting children and adults in developing countries, including Ethiopia.¹ It results in the frequent passage of bloody mucoid stools, abdominal cramps, and tenesmus caused by ulceration of the intestinal epithelium. Such extensive damage of the intestinal epithelial tissues leads to malabsorption of nutrients including vitamin A. Even though we did not see significant difference in serum retinol levels in patients with and without shigellosis, a study in Bangladesh found low retinol concentrations in children with severe shigellosis.³²

Intestinal parasitism occurs widely throughout Ethiopia.³³ In line with this, we found a high prevalence of intestinal parasites in diarrheic patients included in the present study. Earlier data suggest that intestinal parasite infections can affect the nutritional status of infected people by modifying the key stages of food intake, digestion and absorption.³⁴ Abnormalities in the mucosa of intestinal tract were observed in children infected with *Ascaris lumbricoides* by jejunal biopsy, which disappeared rather rapidly after deworming.³⁵ Trophozoites of *Giardia lamblia* were shown to damage the brush borders of enterocytes and impair the activity of mucosal enzymes, particularly the disaccharidases, causing carbohydrate and fat malabsorption.³⁶ The later has a direct association with absorption of vitamin A, since products of fat digestion are essential for efficient solubilization of retinol in the aqueous intestinal milieu.

Impaired bioavailability of vitamin A in adults and children with diarrhoea has been reported in Zambia.³⁷ It is worth mentioning that clinical trials of oral, high dose vitamin A supplementation for HIV-infected children suggest that vitamin A reduces diarrheal morbidity and improves immune status.³⁸ Furthermore, a study of vitamin A supplementation in Tanzanian HIV infected pregnant and lactating woman showed increased serum vitamin A levels in their infants at 6 weeks and 6 months and decreased the prevalence of VAD.³⁹ A meta-analysis on the effect of vitamin A supplementation on morbidity and mortality from infectious disease indicated reduction in deaths from diarrheal disease by 39%, from respiratory disease by 70%, and from other causes of death by 34%.⁴⁰ These indicate that adequate supply of vitamin A, either through supplementation or adequate diet, has a major

role in preventing morbidity and mortality.

An association between the acute phase response to infection or inflammation and alterations in the serum concentration of retinol has been demonstrated previously.^{41,42} The variations in the serum levels retinol are brought about by changes in the concentration of specific tissue proteins that are controlled by cytokines. Although, the changes are generally believed to be beneficial aspects of the early acute-phase response, the assessment of status for micronutrients is particularly difficult, since plasma concentration may bear little relationship to tissue status.⁴¹ Therefore, simultaneous assessment of acute phase proteins such as CRP together with retinol may give a clearer picture on the interpretation of serum levels. A limitation of the present study was the failure to include parameters for the acute phase response or inflammation. Inclusion of such parameters may help to delineate whether the observed values of serum levels of vitamin A in diarrheic patients with or without HIV co-infection versus controls were either due to the acute phase response or due to micronutrient status. In addition, inclusion of data on dietary intake of the patients and clinical evaluation for specific signs of the deficiency may provide useful information to better explain the situation of vitamin A deficiency in the study population.

In conclusion, vitamin A deficiency is a severe public health problem in Gondar, Ethiopia, among diarrheic patients irrespective of HIV co-infection. Intervention programmes involving health and nutrition education and supplementation of vitamin A might help in reducing morbidity in diarrheic patients. Further in-depth studies are needed to substantiate these findings.

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References

1. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003; 81:197-204.
2. Joint United Nations Program on HIV/AIDS/ World Health Organization (UNAIDS/WHO). AIDS epidemic update. Geneva: UNAIDS/WHO, 2005.
3. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003; 81:197-204.
4. Thomas, PD, Forbes A, Green J, Howdle P, Long R, Playford R, Sheridan M, Stevens R, Valori R, Walters J, Addison G, Hill P, Brydon, E. Guidelines for the investigation of chronic diarrhea. *Gut* 2003; 52(Suppl V): V1-V15.
5. World Health Organization. Provisional WHO clinical case definition for AIDS. *Wkly Epidemiol Rec* 1986; 10: 303-306.
6. Black R. Micronutrient deficiency-an underlying cause of morbidity and mortality. *Bull World Health Organ* 2003; 81: 79.

7. Scrimshaw NS, Taylor CE, Gordon JE. Interactions of nutrition and infection. Geneva, World Health organization, 1968.
8. Sommer A, West KP. Vitamin A deficiency: health, survival and vision. Oxford, Oxford University Press, 1996.
9. Ward BJ, Humphrey JH, Clement L, Chaisson RE. Vitamin A status in HIV infection. *Nutr Rev* 1993;13:157-66.
10. Semba RD, Waleska TC, Graham NMH, Cohn S, Vlahov D. Vitamin A deficiency and wasting as predictors of mortality in human immunodeficiency virus-infected injection drug users. *J Infect Dis* 1995; 171:1196-202.
11. Ministry of Health. AIDS in Ethiopia. 5th ed. Disease Prevention and Control Department, Ministry of Health, Addis Ababa, Ethiopia, 2004.
12. Chesebrough M. Medical laboratory manual for tropical countries. Volume 2, Microbiology. Saunders, London, 1987.
13. World Health Organization. Basic laboratory methods in medical Parasitology. World Health Organization, Geneva, Switzerland, 1991.
14. Arroyave G, Chichester CO, Flores H, Glover J, Mejia L, Oslen JA, Simpson KL, Underwood BA. Biochemical methodology for the assessment of vitamin A status: a report of the International Vitamin A Consultative Group. Washington DC, Nutrition Foundation, 1982.
15. Sommer A, Davidson FR. Assessment and control of vitamin A deficiency: the Anancy Accords. *J Nutr* 2002; 132:2845S-2850S.
16. Colebunders RL, Latif AS. Natural history and clinical presentation of HIV-1 infection in adults. *AIDS* 1991; 5(Suppl. 1):S103-S112.
17. Kelly P, Musonda R, Kafwembe E, Kaetano L, Keane E, Farthing MJG. Micronutrient supplementation for the AIDS Diarrhea-wasting syndrome in Zambia: a randomised controlled trial. *AIDS* 1999; 13:495-500
18. Coodley, G. O., M. K. Coodley, H. D. Nelson, and M. O. Loveless. Micronutrient concentrations in the HIV wasting syndrome. *AIDS* 1993; 7:1595-1600.
19. Hanekom WA, Potgieter S, Hughes EJ, Malan H, Kessow G, Hussey GD. Vitamin A status and therapy in childhood pulmonary tuberculosis. *J Pediatr* 1997;131(6): 925-957.
20. Moodley D, Moodley J, Coutsoudis A, Coovadia HM, Gouws E. Vitamin A levels in normal and HIV-infected pregnant women. *S Afr Med J* 1998; 88:1029-1032.
21. Rwangabwoba JM, Fischman H, Semba RD. Serum vitamin A levels during tuberculosis and human immunodeficiency virus infection. *Intern J Tuberc Lung Dis* 1998; 2:771-773.
22. Mugusi FM, Rusizoka O, Habib N, Fawzi W. Vitamin A status of patients presenting with pulmonary tuberculosis and asymptomatic HIV-infected individuals, Dar es Salaam, Tanzania. *Intern J Tuberc Lung Dis* 2003; 7:804-807.
23. Stephensen CB, Alvarez JO, Kohatsu J, Hardmeier R, Kennedy JI, Gammon RB. Vitamin A is excreted in the urine during acute infection. *Amer J Clin Nutr* 1994; 60:388-392.
24. Alvarez JO, Salazar-Lindo E, Kohatsu J, Miranda P, Stephensen CB. Urinary excretion of retinol in children with acute diarrhea. *Am J Clin Nutr* 1995 61(6):1273-1276.
25. Fleck A, Myers MA. Diagnostic and prognostic significance of the acute phase proteins. In: Gordon AH, Koj A, Eds. *The Acute Phase Response to Injury and Infection*. Amsterdam: Elsevier, 1985:249-271.
26. Getahun Z, Urga K, Ganebo T, Nigatu A. Review of the status of malnutrition and trends in Ethiopia. *Ethiop J Health Dev* 2001; 15:55-74.
27. Semba RD. The role of vitamin A and related retinoids in immune function. *Nutr Rev* 1998; 56:S38-S48.
28. Semba RD, Graham NMH, Caiaffa WT, Margolick JB, Clement L, and Vlahov D. Increased mortality associated with vitamin A deficiency during human immunodeficiency virus type 1 infection. *Arch Intern Med* 1993; 153:2149-2154.
29. Semba RD, Miotti PG, Chiphangwi JD, Liomba G, Yang LP, Saah AJ, Dallabetta G, Hoover DR. Infant mortality and maternal vitamin A deficiency during human immunodeficiency virus infection. *Clin Infect Dis* 1995; 21:966-972.
30. Semba RD, Miotti PG, Chiphangwi JD, Saah AJ, Canner JK, Dallabetta GA, Hoover DR. Maternal vitamin A deficiency and mother-to-child transmission of HIV-1. *Lancet* 1994; 343:1593-1597.
31. Nduati, RW, John GC, Richardson BA, Overbaugh J, Welch M, Ndinya-Achola J, Moses S, Holmes K, Onyango F, Kreiss JK. Human immunodeficiency virus type 1-infected cells in breast milk: association with immunosuppression and vitamin A deficiency. *J Infect Dis* 1995; 172:1461-1468.
32. Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. *Am J Clin Nutr* 1997; 66:464S-77S.
33. Mitra AK, Alvarez JO, Wahed MA, Fuchs GJ, Stephensen CB. Predictors of serum retinol in children with shigellosis. *Am J Clin Nutr* 1998; 68:1088-1094.
34. Kloos H, Tesfayohannes TM. Intestinal parasites in Ethiopia. In: Kloos H, Zein AZ, eds. *The ecology of health and disease in Ethiopia*. Oxford: Westview Press, 1993:223-235.
35. Rosenberg IH, Bowman BB. Impact of intestinal parasites on digestive function in humans. *Feder Proc* 1984; 43:246-250.
36. Tripathy K, González F, Lotero H, Bolaños O. Effects of *Ascaris* infection on human nutrition. *Amer J Trop Med Hyg* 1971; 20: 212-218.
37. Crompton DWT. Nutritional aspects of infection. *Trans Roy Soc Trop Med Hyg* 1986; 80:697-705.
38. Kelly P, Musuku J, Kafwembe E, Libby G, Zulu I, Murphy J, Farthing MJ. Impaired bioavailability of vitamin A in adults and children with persistent diarrhoea in Zambia. *Aliment Pharmacol Ther.* 2001; 15:973-9.
39. Coutsoudis A, Bobat RA, Coovadia HM, Kuhn L, Tsai WY, Stein WA. The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. *Am J Public Health* 1995; 85:1076-1081.
40. Baylin A, Villamor E, Rifai N, Msamanga G, Fawzi WW. Effect of vitamin supplementation to HIV-infected pregnant women on the micronutrient status of their infants. *Eur J Clin Nutr* 2005; 59:960-8.
41. Glasziou PP, Mackerras DEM. Vitamin A supplementation in infectious diseases: a meta-analysis. *Br Med J* 1993; 306:366-370.
42. Beisel WR. Infection-induced depression of serum retinol - a component of the acute phase response or a consequence? *Am J Clin Nutr*; 1998; 68(5): 993-994.
43. Stephensen CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. *Am J Clin Nutr* 2000; 72(5):1170-1178.