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VITAMIN A LEVELS IN HIV/AIDS
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E. M. KAFWEMBE, P. KELLY and P. NGALANDE

ABSTRACT

Objective: **To study the correlation of vitamin A concentrations in patients with AIDS, HIV positive symptom free and HIV negative symptom free men and women.**
Design: **A cross-sectional study.**
Subjects: **Male and female volunteers aged between 15 and 60 years willing to undergo an HIV-test.**
Setting: **Participants came from different backgrounds within the city of Ndola. Some were urban while others were peri-urban dwellers. They were included in the study only if they were willing to undergo the HIV test regardless of their place of residence.**
Main outcome measures: **After obtaining consent blood samples were taken from the participants using needle and syringe. Whole blood was used to measure haematological indices while serum was used to measure vitamin A concentrations and HIV status.**
Results: **One hundred and thirty five participants were recruited for the study. Vitamin A was analysed in eighty seven HIV negative symptom free, forty one HIV-positive symptom free and seven AIDS cases. There was a significant difference ($p<0.05$) in the variance of vitamin A levels in the three groups. Vitamin A deficiency is defined as blood concentrations below 30 µg/dl. Using this cut-off point, the Odds Ratio for deficiency if HIV-positive was found to be 6.3 (2.5, 16.7 $p<0.0001$). The Odds Ratio for HIV and serum vitamin A deficiency was approximately the same for males and females. There was a modest correlation between vitamin A concentrations and haemoglobin ($r=0.34$, 95% CI 0.18, 0.48, $p<0.0001$).**
Conclusion: **Vitamin A concentration is lowered in HIV infection. The depletion of vitamin A seems to increase with progression of the infection leading to AIDS disease. Whether regular supplementation of vitamin A to the HIV infected individual can lead to a delayed progression to AIDS needs to be explored.**

INTRODUCTION

AIDS is characterised by severe weight loss, probably arising from inadequate food intake because of anorexia and/or malabsorption. Micronutrient deficiency in AIDS has been widely reported(1). Malnutrition also manifests as deficiency in micronutrients such as zinc, vitamin A, iron, iodine and trace elements. Apart from occurring as a consequence of reduced intake, micronutrient deficiency can also be due to increased tissue utilisation(2) and urinary excretion(3,4). It is important to determine whether vitamin A deficiency occurs early in HIV-disease. In our earlier study in Zambia, we discovered that 67% of AIDS patients in the study were vitamin A deficient(5). We decided to study the concentrations of vitamin A in AIDS patients as well as HIV-positive and negative symptom free men and women in order to determine the correlation in vitamin A concentrations in the three groups.

MATERIALS AND METHODS

Study design: Participants were recruited through Hope Humana, an organisation that offers voluntary counselling and testing (VCT) services to those who wish to have an HIV test in Zambia. Hope Humana have been involved in this service for many years and have a listing of both HIV-positive and negative candidates who have passed through their offices for counselling. Hope Humana import second hand clothes and shoes for sale to raise funds to pursue developmental projects in Zambia. They have several shops in the country and employ many people in these businesses. In Ndola alone, they have at least six shops and warehouses with a workforce of well over 100. Most of these employees live in the peri-urban areas of Ndola town. Using Hope Humana as the entry-point the research team were able to interview these clients. The purpose of the study was clearly explained to them. Consent was obtained and counselling given before blood samples were taken. Participants who were on multivitamins or prolonged drug treatment were excluded from the study. Apart from those classified to be AIDS patients(6), only symptom-free HIV positive and negative men and women were recruited. Those who had suffered any illness including diarrhoea in the previous month were excluded. Ethical clearance for the project was obtained from the Tropical Diseases Research Centre ethical committee.

Chemicals and solvents: Retinyl acetate and retinol were procured from Sigma Co LTD, South Africa, n-hexane and HPLC water were purchased from British Drug House (BDH) UK while methanol was purchased from Anatech, South Africa.

Blood collection: Blood was collected by vein puncture using needle and syringe into two containers: one millitre was transferred into an EDTA bottle for analysis of haemoglobin and cell counts on the same day and two millitres into a plain tube for HIV-testing and the analysis of vitamin A. Samples were kept in a cool box and protected from light for transportation to the laboratory. Blood samples in the plain tube were centrifuged and the recovered serum samples were stored at -20°C until analysis for vitamin A by High Performance Liquid Chromatography (HPLC).

HIV testing: All the blood samples were tested for HIV status. HIV testing was performed initially using a rapid test (Capillus 1& 2, Trinity Biotech, Ireland) and confirmed by Western Blot.

Haemoglobin estimation: Haemoglobin estimations were performed on a haemoglobinometer (Isoton, Zapoglobin)(7).

HPLC: The HPLC instrumentation used consisted of a Pye Unicam pump (PU 4050), a Pye Unicam UV detector (PU 4025), a Pye Unicam integrator (PU 4010) and a Waters 717 plus autosampler. A supelcosil column (LC 18, 25cm x 4.6mm, 5 µm) was used for elution.

Extraction procedure: An aliquot of serum (200 µl) was put in a clean screw capped test tube (6cm x 1.5cm). To this 200 µl of ethanol and a known amount of internal retinyl acetate standard were added. After vortex mixing for about 15 seconds, 500 µl of n-hexane was added and the contents vortex mixed for 45 seconds. The mixture was then centrifuged at 3000 rpm for two minutes before the top n-hexane layer was separated to a separate tube. A second extraction using 500 µl n-hexane was performed on the same tube sample and the top n-hexane layer separated to the corresponding tube after vortexing and centrifuging as above. The double extracted n-hexane was then evaporated to dryness under a stream of nitrogen gas and the residue re-dissolved in 200 µl of Dichloromethane:Propanol (4:1 v/v) solution. By means of the auto-sampler, 25 µl of this solution was injected onto the column and elution performed. The sample was carried through the column at a flow rate of 2 ml/min and the elution was detected at 325nm. A methanol/water solvent (98:2) was used.

Standardisation: Both retinol and retinyl acetate were purified by HPLC before use. Stock standards were prepared in ethanol. Working standards were prepared by using $E^{\%}$ values (retinol: 1835 at 325nm and retinyl acetate: 1550 at 326nm). Retinol and retinyl acetate standard concentrations and their retention times were entered in the integrator memory to allow for internal calculations of the unknown sample extracts and the results automatically calculated.

Statistical analysis: All results were entered in the computer using Dbase soft-ware. Epi 6 software was used to calculate the mean, median, and correlation coefficients (expressed with 95% confidence intervals).

RESULTS

A total of 135 participants were recruited for the study. Using the GrusGal-Wallis test, vitamin A concentrations were compared in three categories of the population, eighty seven HIV-negative symptom free men and women, forty one HIV positive symptom free men and women and seven men and women with symptoms attributed to AIDS. Serum retinol concentration was reduced in HIV disease and further reduced in AIDS (Table 1).

Table 1

Vitamin A levels in a Zambian urban population in relation to HIV status

	No.	Mean	Median	IQR	P value
HIV neg	87	41.7	39.0	32.9-45.3	0.002
HIV pos	41	35.1	32.5	24.4-43.4	
AIDS	7	26.2	21.7	13.0-34.9	

Vitamin A deficiency can be defined as a blood concentration below 30 µg/dl (1.05 µmol/l). Using this cut-off point the Odds Ratio for deficiency if HIV-positive was found to be 6.3 (95% C.I 2.5, 16.7 p<0.0001). When serum vitamin A concentration was analysed as a continuous variable, the difference in HIV-positivity and HIV-negativity for both sexes was striking (p=0.02 for women and p=0.05 for men). There was a modest correlation between vitamin A concentrations and Haemoglobin (r=0.34, 95%CI 0.18, 0.48, p<0.0001). Table 2 gives the vitamin A and haemoglobin concentrations of the seven AIDS patients in the study.

Table 2

Haemoglobin and serum vitamin A concentrations of seven AIDS patients

Patient No.	Hb conc. (g/dl)	Vit. A conc. (µg/dl)
1*	8.10	12.81
2	10.20	21.78
3*	10.00	13.87
4	14.40	33.93
5*	7.90	13.00
6	14.20	61.92
7*	7.30	26.16

By the end of the three months follow up, four of the AIDS patients died (shown by the star). As can be seen all had haemoglobin concentrations below 10.0g/dl and vitamin A levels below 30 µg/dl. The survivors had haemoglobin concentrations above 10.0g/dl. Three of the four patients who died had serum vitamin A concentrations below 20 µg/dl indicating severe deficiency

DISCUSSION

Vitamin A concentration in serum was lower in HIV-infected than the non-infected (p<0.05). But individuals with the full-blown disease had even lower concentrations compared to symptom free HIV-infected, and symptom free non-infected individuals. The reason for the lowering of the concentrations with progression to the full-blown disease may be due to the depletion of vitamin A due to a number of factors. Firstly, during acute infection the body responds by producing acute phase proteins to repair the resulting tissue damage. If this challenge is prolonged, the over utilisation of nutrients in this synthesis results in deficiency. Decreased vitamin A and E concentrations have been reported during infection(8). HIV-infected individuals are prone to infection because of their weakened

immunity. Secondly, micronutrients including vitamin A are lost through urinary excretion during infection and diarrhoea may compound the depletion. Thirdly, apart from the acute phase response, infection induces release of reactive oxygen species called free radicals. Ironically, it has also been reported that HIV replication in cell cultures is stimulated by these reactive oxygen species(8). If not quenched, free radicals can cause further damage to tissue by reacting with them. Most vitamins, including vitamin A are good quenchers of free radicals. In HIV-infection, therefore, a lot of vitamin A and other micronutrients are used up in this process. It has been found that due to prolonged infection, the demand for vitamin A and other micronutrients in HIV/AIDS is so high that the intake of these at levels recommended for the general population does not appear to be adequate(10).

It seems likely that with prolonged infection with the virus there is increased utilisation of vitamin A in the body's effort to strengthen the immune system and quench free radicals. With time the vitamin A is depleted and this results in even weaker immunity. The frequent infections that follow further lower the vitamin A concentrations. Concentrations of vitamin A in HIV-infection could therefore be used as an indicator of the disease process. Our earlier study in Zambia showed vitamin A concentration to be a predictor of death. Studies conducted elsewhere have shown that low serum retinol (<30 μg/dl) levels are associated with a four-fold increase in mortality(11). In South Africa, vitamin A deficiency was reported to be associated with HIV-infection(12). Another study in Malawi showed serum retinol concentration among pregnant HIV-infected women to be inversely related to maternal mortality as well as mortality of their infants during the first year of life(13). It was found that mortality dropped from 93% in infants from women with serum vitamin A concentration <10 μg/dl to 14% in infants of mothers with normal vitamin A levels.

Several studies have shown that micronutrient intake is an important determinant of progression of the HIV disease(14,15). Whether regular supplementation of vitamin A to the HIV-infected individual can lead to a delayed progression to AIDS needs to be explored.

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