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COMPARATIVE ANALYSIS OF MICRONUTRIENTS STATUS OF HIV INFECTED AND HIV NON- INFECTED SUBJECTS ATTENDING THREE SELECTED HOSPITALS IN KANO METROPOLIS, KANO STATE, NIGERIA

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ABSTRACT
Comparative studies on serum levels of micronutrients (iron, iodine, Vitamin A and β – carotene) were carried out among HIV negative and people living with HIV and AIDS attending three hospitals in Kano, namely Murtala Mohammed Specialist Hospital, Aminu Kano Teaching Hospital and Infectious Diseases Hospital. HIV infection was confirmed using indirect solid phase enzyme immunoassay technique of Immunocomb II HIV 1 and 2 Bi spot kits. Serum micronutrients levels of 270 subjects were detected using colorimetric and spectrophotometric techniques. Results obtained showed that serum vitamin A and β-carotene concentrations decrease with increase in age and were higher in males than females. The mean serum concentrations (in µg/dl) of iron, iodine, vitamin A and β- carotene were found to be 99, 1.53, 39.4, 45.98 for HIV negative subjects; 62.6, 1.54, 20.16, 19.2 for HIV positive subjects and 28.8, 0.9, 16.1 and 18.0 for AIDS subjects respectively. Deficiency of iron, vitamin A and β-carotene were found to be more common among people living with HIV and AIDS when compared with HIV negative subjects at 5% level of significance with AIDS patients having the lowest value ($p<0.05$). This could be due to malabsorption, altered metabolism, reduced food intake and/or infection in HIV infected persons. Iodine level of these patients was found to be normal (1.54 and 0.9 µg/dl). The results imply that HIV infection affects the serum levels of iron, vitamin A and β-carotene by reducing their levels; hence, dietary supplementation with them could be useful in maintaining good health in HIV infected subjects and reducing mortality.
Key words- AIDS, HIV, Kano, Micronutrients, Serum.

INTRODUCTION

Micronutrients are required in the human diet in only milligram or micrograms quantities per day. They include vitamins such as vitamin A, D, E, K, B and C and minerals such as Iron, iodine, zinc, chromium, potassium and chlorine (Lehninger, 1987).

In recent years, the importance of nutrition in human health has received growing attention. Therapeutic and preventive supplementation with vitamins has been used successfully for a long time for many clinical conditions; this includes vitamin A for maintenance of vision, beta carotene in erythropoetic protoporphyria, Vitamin C in Scurvy, niacin in pellagra and several others (Omenn *et al.,* 1996). *In vitro* and animal studies have shown immunostimulatory and anticancer properties of several micronutrients leading to several, large epidemiological trials of micronutrients supplementation (Blot *et al,* 1992). Micronutrients were also involved in strengthening the immunity and in fighting infection (Hennekens, 1996).

HIV infection is caused by human immunodeficiency virus, a lentivirus within the family Retroviridae (Prescott *et al.,* 1999). It causes progressive impairment of the body's cellular immune system leading to increased susceptibility to infection and tumors and the fatal condition Acquired Immune Deficiency syndrome, (AIDS) (Cheesbrough, 1990).

At the moment there is no complete cure for AIDS, making research very important in the field. Antiretroviral drugs are used to reduce the viral load, disease symptoms and opportunistic infections. Unfortunately; these drugs are unaffordable by many victims and are extremely toxic with many side effects such as loss of appetite, blotting, diarrhea, lactic acidosis, pancreatitis, lipoatrophy, peripheral neutropathy, fatigue, sexual dysfunction, kidney stones, nausea and vomiting (Hennekens, 1996). As a result, alternative ways of managing HIV infection are needed. Randomized controlled trials are therefore needed to clarify the relationship between potentially beneficial micronutrients and HIV progression and transmission. If success with one or a combination of micronutrients can be proven, then developing countries could have affordable, cost-effective and safe public health interventions at hand.

Assessment and correction of nutritional status in HIV infection are being recognized as an important part of comprehensive care of persons infected with HIV (Neera, 2002). Determination of nutritional status of the patient with view to supplementation of deficiencies may culminate into strengthening their immune system; hence the need to have a base line data on the micronutrient level in HIV / AIDS subjects.

This study was aimed at determining and comparing the serum levels of some micronutrients (Vitamin A, β – Carotene, iron and Iodine) in normal subjects

and people living with HIV and AIDS, with a view to evaluating their possible role in HIV complication.

MATERIALS AND METHODS

Sampling site

The study was carried out between January 2008 and January 2009, in three (3) selected hospitals: Murtala Muhammad Specialists Hospital, Aminu Kano Teaching Hospital and Infectious Disease Hospital Kano state, Nigeria.

Ethical consideration

With due approval of the ethical committee of the hospital, blood samples for the study were collected from informed and consented confirmed HIV positive subjects, AIDS patients and HIV negative individuals.

Study population

The study sample consisted of 270 subjects of either sex with ages ranged from 15- 70 years. One hundred subjects were HIV negative subjects, 100 HIV positive subjects and 70 were subjects with clinical diagnosis of AIDS (CD4 counts ≤ 200 cells/µl of blood).

Blood sample collection and HIV Screening

Five milliliters (5 ml) of whole blood was collected using standard venipuncture method and dispensed into a test tube. The blood samples were centrifuged at 2500rpm for 10 minutes. The supernatant / serum portions were decanted for use and the lower portions discarded. All the sera collected were screened for the presence of HIV using indirect solid phase enzyme immunoassay technique of Immunocomb test according to manufacturer's specifications (Immunocomb II, HIV 1 and 2 BI spot kits PTE LTD India).

Micronutrient assays

Serum iron levels were determined according to the procedure of Annino and Giese (1976), and Sule (2001). The method involves the liberation of iron from proteins and subsequent coupling of the reduced iron with ferozine to give a purple colored complex. Absorbance of the standard (ferrous ammonium sulfate solution) and each test (serum) sample was read from the colorimeter at 560nm, setting the blank (iron free water) at Zero. The iron content of each sample was calculated using the formula:

$$\text{Serum iron concentration } \mu g/dl = \frac{ab.t}{ab.s} \times K \times \frac{1}{1.015}$$

Where:

ab.t = absorbance of the test

ab.s = absorbance of the standard

K = concentration of the standard

Error! Reference source not found. = volume correction factor

Levels of iodine in serum were determined using dry ashing method of detecting protein bound iodine as described by Brown *et al.,* (1953). The method is based on the catalytic action of iodine on the oxidation arsenite by ceric sulfate in which the yellow Ce^{4+} is converted to colorless Ce^{3+} ion. The absorbance of the standard (potassium iodine solution) and each test (serum) samples was read at 420nm in a spectrophotometer using 10mm path length, setting the blank (water) at zero.

Carr - Price method described by Stroev and Makarova (1989) was used for determining vitamin A and β -carotene in serum. This method involves the precipitation of proteins with ethanol and the vitamin A and carotenes extracted into light petroleum. After reading the intensity of the yellow color due to the carotenes colorimetrically at 440nm, the light petroleum was evaporated and the residue dissolved in chloroform. Carr- price reagent was added and the amount of blue color produced read colorimetrically at 440nm using light petroleum as blank. Since carotenes also give some color a correction for this is made in order to obtain that due to vitamin A (retinol) present. Calibration curves were prepared using series of absorbance obtained colorimetrically from several dilutions of standard solutions of β-carotene and retinol.

The serum carotene concentration obtained was used to read the amount of color due to carotene from carotene correction curve. The colorimeter reading due to vitamin A was obtained by subtracting the color due to carotene from actual colorimeter reading and the serum retinol was read from retinol standard curve.

Statistical analysis

Statistical Analysis was performed using SAS software. The level of significance was fixed at 0.05.

RESULTS

In HIV negative subjects screened, age group 18 – 22 years had the highest mean serum levels of vitamin A and β-carotene of 52µg/dl and 90µg/dl respectively. Age group 48 years and above had the lowest serum levels of 28 and 31µg/dl for vitamin A and β-carotene respectively as shown in Table 1. Thus, the serum levels of these micronutrients decreases with increase in age. Iron serum levels were highest in age group 33-37 (100 µg/dl) and lowest for age group 18- 22 and 43-47 years (60 µg/dl). Serum iodine level of these subjects was highest in age group 23-27(1.9 µg/dl) years and lowest for the age group 33 -37 years (0.7µg/dl). Thus iron and iodine serum levels of the subject depict an irregular pattern with respect to age.

Similarly in HIV positive group vitamin A and β-carotene levels were highest in age group 18-22 years with 35 and 28 µg/dl, respectively. The lowest serum levels were observed in age group 48 years and above. Iron and iodine level of these subjects also depicts an irregular pattern with respect to age as shown in table 2. Mean serum micronutrients level of AIDS subjects are presented in table 3. Among the AIDS subjects, mean micronutrient concentration were highest among the 23-27 years age bracket except for iodine. The least mean concentrations of vitamin A, β-carotene and iron were lowest among the subjects age 48 and above. Mean serum micronutrients levels (Iron, Iodine, vitamin A, and β-Carotene) in relation to the sex of the subjects are presented in Table 4. Mean serum vitamin A and β-carotene levels were higher in males than in females ($P<0.05$). Iron and iodine serum levels of the subjects were statistically similar in males and females ($P> 0.05$).

Results of serum micronutrients levels in HIV negative, HIV positive and AIDS subjects are presented in Table 5. Mean serum levels of iron vitamin A and β-carotene were significantly higher (P< 0.05) in HIV negative subjects with values of 99µg/dl, *39.4*µg/dl and *45.98*µg/dl, respectively. Significantly lower serum levels of these micronutrients were observed in HIV positive and AIDS subjects (P< 0.05). However, no statistical difference was observed in analysis of serum iodine levels between HIV positive and HIV negative subjects (P>0.05).

Table 1: Mean ±SD values of Serum Micronutrients concentrations (µg/dl) in HIV negative subjects with respect to age

Age group (years)	**Number of samples analysed (n=100)**	**Vitamin A**	**β-Carotene**	**Iron**	**Iodine** Error! Reference source not found.
18-22	11	52±7.8	90±9.8	60±15.5	0.8±0.3
23-27	13	40±6.1	52±3.4	98±21.1	1.9±0.7
28-32	19	35.8±6.3	45±10.3	76±8.6	1.3±0.3
33-37	20	32±7.9	45.6±9.2	100±20.1	0.7±0.2
38-42	12	31.6±5.8	36±15.1	59±9.1	1.0±0.4
43-47	12	30.5±6.8	31±8.3	60±4.1	0.9±0.5
48 - above	13	28±7.9	31±7.5	63±8.1	1.7±0.6

Table 2 : Mean ±SD values of Serum Micronutrients concentration (µg/dl) in HIV Positive Subject with respect to Age

Age group (years)	**Number of samples analysed(n=100)**	**Vitamin A**	**β-Carotene**	**Iron**Error! Reference source not found.	**Iodine** Error! Reference source not found.
18-22	2	35±7.8	28±4.2	52±9.2	0.9±0.1
23-27	10	30.3±3.1	20±2.6	60±15.6	2.0±0.7
28-32	19	31.6±6.8	19±5.6	58±15.0	1.4±0.5
33-37	23	18.3±7.1	19.8±2.1	67±16.2	0.8±0.7
38-42	20	16.6±6.0	18±3.2	43±9.1	1.1±0.6
43-47	16	17.8±7.3	17.3±3.4	60±15.4	0.8±0.4
48 - above	10	16±6.1	15±2.3	40±8.9	1.8±0.6

Table 3: Mean ± SD values of Serum Micronutrients concentration (µg/dl) in AIDS Subjects with respect to Age

Age group (years)	**Number of samples analysed (n=70)**	**Vitamin A** Error! Reference source not found.	**β-Carotene** Error! Reference source not found.	**iron**Error! Reference source not found.	**Iodine** Error! Reference source not found.
18-22	0	-	-	-	-
23-27	2	20±5.0	20±3.6	30±5.2	1.1±0.1
28-32	9	17±4.3	15±1.8	25±3.3	0.8±0.08
33-37	11	17±4.1	16±2.1	29±4.1	0.5±0.06
38-42	22	13±3.4	12±3.8	26.8±3.0	0.2±0.06
43-47	16	11.3±2.8	9.6±1.8	27±2.9	0.16±0.03
48 - above	10	10.8±3.9	10.1±1.9	18±2.2	1.3±0.3

Table 4: Mean ± SD values of Serum Micronutrients concentration in HIV Negative, HIV positive and AIDS Subjects with respect to sex

Micronutrients $\mu g/dl$	**sex**	**HIV negative (F=50,M=50)**	**HIV positive (F=50,M=50)**	**AIDS (F=40,M=30)**
Vitamin A	Male	48.3±7.4*	23.12±6.7*	17.4±3.8*
	Female	30.5±6.9	17.8±5.9	14.8±4.2
β-carotene	Male	51.16±14.6*	22.4±2.5*	19.5±4.3*
	Female	40.8±15.3	16.0±2.1	16.5±3.9
Iron	Male	100±21.3	62.0±14.8	28.0±3.6
	Female	98±19.9	63.0±15.4	29.6±2.8

Iodine	Male	1.4±0.5	1.59±0.6	1.0±0.18
	Female	1.66±0.6	1.49±0.58	0.8±0.09

*= significant difference (p<0.05); F=female, M= male

Micronutrients	**HIV negative (n=100)**	**HIV positive (n=100)**	**AIDS Patients (n=70)**
Iron (µg/dl)	99 ± 20.99[a]	62.6±15.3[b]	28.8±3.1[c]
Iodine(µg/dl)	1.53±0.5[a]	1.54±0.61[a]	0.9±0.095[a]
Vitamin A(µg/dl)	39.4±7.3[a]	20.16±6.6[b]	16.1±4.1[b]
β- carotene(µg/dl)	45.98±15.4[a]	19.2±2.1[b]	18.0±1.3[b]

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Table 5: n ± SD values of Serum Micronutrient Concentration in HIV Negative, HIV Positive and AIDS Subject Values with the same superscript in a row are not significantly different (P>0.05)

DISCUSSION

Serum micronutrient levels are used to characterize micronutrients deficiencies which are influenced by factors such as gender, time of the day of measurement, acute infection, liver disease, experimental error and recent intake (Neera, *et al.,* 2002). There may be interaction between micronutrients and concomitant antiretroviral drug therapy making generalization of findings to diverse population difficult (Neera, *et al.,* 2002).

Results obtained with respect to higher serum vitamin A (52µg/dl) and β - carotene (90µg/dl) at the age of 18 - 22 years, could be due to high metabolic activity at the age of 19 - 22 years, which tends to decrease with age (Lehninger, 1987). This observation is similar to the report of IVACG (1976) who reported that the peak serum concentration of some macronutrients including vitamin A is attained at the age of 18 -21 years in healthy and well fed subjects. The higher serum concentration of vitamin A and β - carotene in males (48.3 and 51.16 µg/dl) than in females (30.5 and 40.8 µg/dl) agrees with a previous report by Sara and James, 1990, who reported a mean serum vitamin A concentration in males and females as 800IU and 600IU respectively. This might be attributed to the fact that the daily requirement and average weight of males is higher than that of females; hence more storage in the serum of males (Lehninger, 1987).

Iron and iodine are essential elements for the synthesis of hemoglobin and thyroid hormones, respectively. Fluctuation of serum iron and iodine levels in relation to age and sex obtained in this research could be said to conform with the theory that serum iron and iodine levels are not markedly affected by age and sex (Sood, 1989). However, the lack of significant difference in serum iron levels between males and females observed in this research disagree with the report which says women are especially likely to lack iron than males (Sara and James, 1990). This is because of significant monthly blood loss associated with menstruation in women (Sara and James, 1990).

The serum micronutrient levels (Iron, iodine, vitamin A and β - carotene) of HIV negative subjects screened 99, 1.53, 39.4 and 45.98µg/dl respectively fell within the normal range of 50 - 180µg/dl, 0.05 -2µg/dl, 30-60 µg/dl and 20-200 µg/dl respectively, as previously reported (Annino and Giese, 1989; IVACG, 1976; Stroev and Makarova, 1989). This could be due to the fact that the subjects had adequate dietary intake to meet their daily micronutrients requirements and that they are not suffering from diseases, which could aggravate deficiencies.

A highly significant lower serum iron levels in HIV positive (62.6ug/dl) and AIDS subjects (28.8 µg/dl) in comparison with HIV negative subjects (99 µg/dl) is in conformity with the report by Castaldo *et al.* (1996) which reported iron deficiency in AIDS and some asymptomatic HIV positive subjects. They also noted that HIV infections could be suspected among anemic women living in areas of high HIV prevalence, who do not respond to iron folate supplementation. De Monye *et al.* (1999) was also able to discover the increase in iron stores in bone marrow, muscles, liver and other tissues of HIV infected and AIDS patients, which could account for lower serum iron in the subjects. Several studies have also found that anaemia is associated with HIV disease progression, although it is not only caused by iron deficiency (Moore *et al.,* 1998). The lower serum iron levels observed in this research contradict the study of Donna (1999) who reported higher iron levels in HIV infected subjects than in non - infected subjects.

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Lower serum levels of vitamin A (20.16µg/dl) in HIV positive and AIDS subjects (16.1µg/dl) obtained in this research is in agreement with the reports of Semba *et al.,* 1993. Similar studies by Beach *et al.* (1992) revealed that vitamin A deficiency is common in various stages of HIV infection and the level appears to become lower as the disease progresses. This exactly was obtained in this research, which could be postulated to be due to malabsorption, diarrhoea, gut infection, altered gut barrier function and altered metabolism in HIV infected persons (Niki *et al.,* 1995).

The significant higher β - carotene level in HIV negative subjects (45.98 µg/dl) when compared with HIV positive (19.22 µg/dl) and AIDS subjects (18.0 µg/dl) agrees with the works of Tomaka *et al.* (1994) and Lacey *et al.* (1996). Correlation observed between HIV status and serum vitamin A and β - carotene levels in adult studies by Lacey *et al.* (1996)

found 6.5 fold decrease in serum concentration in HIV positive subjects compared with HIV negative controls and those with AIDS had a 13 - fold decrease. Low serum β -carotene in HIV patients may be due to long standing fat malabsorption caused by villous atrophy and impaired electrolyte function, scavenging of singlet oxygen by β-carotene can also lead to its depletion in the serum (Bendich, 1992).

Based on what was obtained in this work, deficiency of some micronutrients (that is that of iron, vitamin A and β - carotene) was found to be common among HIV positive and people living with AIDS compared to the normal individuals (HIV-negative). This could be postulated due to reduced food intake, poor absorption, changes in metabolism and chronic opportunistic infections in HIV infected subjects (NACP, 2000).

CONCLUSION AND RECOMMENDATION

The findings of this research revealed that the serum levels of iron, vitamin A and β - carotene were significantly lower in HIV positive and AIDS subjects than in HIV negative subjects. This implies that HIV infection leads to the reduction in the serum levels of these micronutrients, therefore dietary supplementation with them could be useful in improving the health of HIV - infected subjects. The serum levels of vitamin A and β -carotene were found to be lower in females than males, and also lower in older than in younger subjects. It can therefore be recommended that any dietary supplementation should be more targeted towards females and older subjects than males and younger ones.

Iodine mainly functions in the formation of thyroid hormone (triiodothyroxine and thyroxine) which function in the control of basal metabolic rate. The fact that there is no significant difference in the levels of iodine in HIV negative and HIV positive patients suggests that HIV infection does not interfere with the level of thyroid hormones (Talwar *et al.,* 1989).

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