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Full Length Research Paper

Non-reliability of Beta-2 Microglobulin and Neopterin as Short-term Immunological Markers of HIV Progression

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ABSTRACT

The conventional markers for HIV monitoring in Nigeria are viral load and $CD4^+$ count. However, studies have reported the reliability of Beta 2 microglobulin (B2M) and Neopterin as effective prognostic markers that are less expensive and very convenient to use. This study was designed to investigate possible role of B2M and Neopterin in HIV patients' management in Nigeria. A case series descriptive study was carried out in a group of Forty (40) HIV seropositive subjects who were yet to commence therapy in order to assess the pattern of changes in the levels of B2M, Neopterin and CD4+ T cell count when compared with levels after subjects were followed up for a period of 3months into therapy. B2M and neopterin were measured by ELISA method and CD4 count by flow cytometry. Concentrations of both Neopterin and B2M correlated significantly with each other ($p < 0.05$), but did not correlate with CD4 count ($p > 0.05$). Moreover, no significant difference was observed in the mean CD4 count and B2M concentration of subjects at baseline and follow-up, but there was a significant difference in the concentrations of Neopterin ($p < 0.05$). With the classification of subjects into the therapy and the non-therapy group, significant differences were observed in the concentration of CD4 count, whereas there were no significant differences in the concentrations of B2M and Neopterin at baseline and follow-up between the group on therapy and the therapy naïve group. This study shows that B2M and Neopterin may not be adequate or reliable in monitoring short term (≤3 months) improvement with therapy in HIV/AIDS.

Keywords: HIV/AIDS, Beta 2 microglobulin, Neopterin, $CD4^+$ count, Viral load

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INTRODUCTION

Human Immunodeficiency Virus (HIV) is a lentivirus that infects vital cells in the human immune system such as $CD4^+$ T cells, macrophages and dendritic cells (Cunningham *et al*., 2010).Since 1980's when HIV was isolated from patients with opportunistic infections and Kaposi sarcoma there are millions of people living with this dreadful virus (Barre-Sinoussi*et al*, 1983; Gallo *et al*,1983 and UNAIDS 2010). It was estimated that no infectious organism has claimed more lives in history than HIV (UNAIDS 2010).

HIV infection may progress to acquired immunodeficiency syndrome (AIDS) at different rates in different individuals, with a spectrum varying from rapid progression to long term non-progression (Gupta and Gupta 2004).Hence the need for reliable marker(s) that can effectively depict the true state of the immune system as well as demonstrate the effectiveness of HIV interventions or therapy.Chandra *et al*., 2005 identified three categories of immunologic markers as having significant correlate to the prognosis of HIV infection. They are the HIV viral load, the CD4 T-cell lymphocyte levels, and plasma levels of soluble markers of immune activation.

The body's acquired immunity system is known to express plasma activation markers such as beta-2 microglobulin, neopterin, and cytokines which are products of the activity of the body's immune cells. These markers are

reported to reflect the immunological activities that take place in the body during infection. In spite of their apparent usefulness, they are not currently widely used clinically for this purpose (Sabin et al. 1994;Peto 1996; Chandra et al. 2005).Moreover, there is paucity of information on the usefulness and reliability of these surrogate markers in sub-Saharan Africa, especially Nigeria.

Previous studies have documented the ability of surrogate markers to predict infection or disease progression, jointly and independently (Anderson et al.1990; Shi et al.1997; Mocroft et al. 1997; Wanchu et al.2004; Gupta et al. 2004 ; Wirleitner et al. 2005).The use of surrogate markers with the conventional CD4 T-lymphocyte count and viral load in the study of HIV/AIDS are the principal biological markers utilized for clinical evaluation. However, measuring CD4 counts and viral load has been observed to be laborious, requiring expensive equipments, separate space and are restricted to specialised laboratory and trained personnel (Fuchs et al., 1991; Chandra et al., 2005).

In Nigeria and in most of sub-Saharan African countries, viral load and $CD4^+$ count are considered as integral part of the standard care in HIV infected individuals but they are not at the reach of majority of patients population living in the rural areas and low-tier towns or cities as the most affected (Forbi et al., 2010).As a result of this,it was posited that immunochemical markers (such as beta 2 microglobulin and neopterin) needed to be investigated for their health monitoring potentials(Helbert and Breuer, 2000; Fuchs et al., 1991).

Beta 2 microglobulin (B2M) is a component of the human major histocompatibility complex (HLA) class I molecule. Fahey et al., 1990 reported that the degree of elevation of serum beta-2-microglobulin correlates well with the extent of disease burden (Pignone et al. 2004 ;Ravotti 2014).Mocroft et al., 1997 illustrates that B2M is an independent prognostic marker for death in patients infected with HIV.Neopterinis a pteridine compound and a metabolite of guanosine. It is released from macrophages and B cells stimulated by interferon gamma and/or interleukin 2. .Neopterin is measured in either serum or urine and the two measurements correlate well (Denz et al., 1990).Its increased concentrations linked to series of pathological states (Fahey et al., 1990).

Changes in the concentrations of neopterin and beta-2 microglobulin closely correlate with one another in HIV infection, thereby reflecting immune system activation (Anton et al.1993;Dunne et al. 1996). Moreover, it is reported that further increase in the concentrations of both markers in the body is seen during the invasion of opportunistic infections. Mulder et al., 1991 reported that measuring serum beta 2-microglobulin was simpler and more possible to assay in every subject compared to CD4 count and viral load. Likewise, other various studies have reported the advantages of beta-2-microglobulin and neopterin over CD4 T cell count and viral load (Brew et al.1990; Fuchs et al.1991; Coberly et al., 1992 ; Krämer et al. 1992 ; Hofmann 1993 ;Boffa1994 ; Boccellari et al.1994 ; Zeller et al. 1996 ; Mocroft et al. 1997 ; Rao et al. 1997).

Although numerous studies in the developed nations have affirmed the reliability and accuracy of neopterin and beta-2-microglobulin as alternative markers for the examination and care of HIV positive individuals because of their correlation and/or advantage over CD4 cell counts, there exist a wide gap in information on the predictive and prognostic value of these markers in developing countries such as Nigeria, in both long and short term basis. This study will evaluate beta-2-microglobulin and neopterin as surrogate markers for monitoring HIV infection in humans on a short term basis

MATERIALS AND METHODS

Instruments for Data Collection: Anonymized questionnaire was used to collect the subjects' socio-demographic characteristics and other relevant information. Blood samples were collected with potassium EDTA bottle.

Study Design: This study is a case series descriptive study with 'before and after' approach. Subjects' beta 2 microglobulin and neopterin concentrations were assayed for (before and after the commencement of therapy) and compared with their CD4 counts within the period of study.

Study Area: The study was conducted at Adeoyo Maternity Teaching Hospital in Ibadan North East Local Government Area, Ibadan, Oyo State, Nigeria.

Ethical approval: Ethical approval was obtained from Oyo State Ministry of Health and Hospital management board that supervised and regulate clinical activities of Adeoyo Maternity Teaching Hospital

Study Subjects: Asymptomatic and symptomatic HIV seropositive individuals who were yet to be placed on antiretroviral therapy and who kept to clinic appointments at the HIV-PEPFAR clinic at Adeoyo Maternity Teaching Hospital, Ibadan, Oyo State, Nigeria were eligible for inclusion in the study.

Sample Size: Based on the documentation that the prevalence of HIV infection in Nigeria is between 2.3 to 3.8% (FMOH 2007) with an average prevalence rate of 3.1% (UNADS/WHO 2008)a minimum of 46 subjects were proposed for the study.

Sampling Technique: Forty (40) confirmed HIV patients who were awaiting anti-retroviral therapy (ART) were recruited into this study after obtaining their consents. 5mls of venous blood was obtained from each participant at the beginning of the study and at three months from the first sample collection.

Inclusion/Exclusion criteria: Only subjects who have been confirmed HIV positive, whose pre- and post-samples/data were captured and who had not been commenced on ARV were included in the study.

CD4 count: This was done by flow cytometry technique (PartecCyFlow Counter®, Partec, Münster ·Germany).20ul EDTA whole blood was collected into Partec test tube (Rohren tube). Then 20 µl of CD4+T antibody were added into the tube. The contents were mixed and incubated in the dark for 15 minutes at room temperature. 800ul of CD4 buffer was gently added into the mixture and mixed gently. Then the Partec tube was plugged on the Cyflow counter and the CD4+T cells were displayed as peaks and interpreted as figures.

Beta-2-microglobulin assay: Beta-2-microglobulin was measured in frozen stored plasma samplesfrom both EDTA and citrate using a commercially available non- competitive

(sandwich) human B2M enzyme linked immunosorbent assay (ELISA) kit [GenWay Biotech Inc, USA].

Neopterin assay: Neopterin in serum was also measured in plasma samples from both EDTA and citrate bottles using commercially available quantitative competitive enzyme linked immunosorbent assay (ELISA) kit[GenWay Biotech Inc, USA].

HIV Staging: Exposure category (i.e. staging) was determined using the World Health Organisation (WHO, 2005) staging criteria.

Data analysis: Statistical software statistical package for social sciences (SPSS Inc., Chicago - version 16.0), was used in the analysis and inference of data collected. Results were expressed as mean ± SDand values were considered statistically significant at $p<0.05$.

Strength of correlation among the immunological markers under study ,i.e. B2M, Neopterin and $CD4^+$ T-lymphocyte count was assessed with use of Spearman's rank correlation coefficient, while the test of significant differences in their concentrations at baseline and follow-up were determined using the Student paired T-test. Changes in concentration of markers/parameters between therapy and non-therapy group were determined using independent sample T-test.

The Pearson correlation test was used to determine the strength of relationship between CD4 cell count and other immunological markers/parameters, while the test of association between markers/parameters and HIV staging was determined using the Chi-square test.

RESULTS

A total of 100 HIV positive subjects were captured for the study out of which only 40 turned up for the follow-up exercise. Two subjects died in the course of the study before the second sample collection was to commence. However, in order to work in tandem with the calculated sample size, an additional 8 pre-samples were drafted for CD4 count, B2M, Neopterin analysis to make a total of 48 initial samples.

From the 48 initial samples, 40 subjects (83.3%) were females, while 8subjects (16.7%) were males.21 of the subjects (43.8%) had only primary education, 15 subjects (31.2%) had secondary education while only 4 subjects (8.3%) had tertiary education. However, 8 (16.7%) subjects had no form of formal education.

The mean age of subjects was 37 ± 14 years with the age range spanning from 7 months to 70 years. Most of the subjects (56.2%) were traders, while 10.4% were not income earners. A fairly large number of subjects (25.2%) were artisans and casual workers of various vocations, while 4.2% were retired pensioners. Only 4.2% were engaged in formal jobs.

Out of the 48 subjects, 25 (52.1%) were Christians, 22 subjects (45.8%) were Muslims, while 1 subject (2.1%) practised both Christianity and Islam. 38 subjects (79.2%) were married, 8 subjects (16.7%) were single, 2 subjects (4.2%) were divorced. However, from the 40 follow-ups, 10 subjects (25%) subjects were still in the asymptomatic/stage I phase of HIV infection, while 34 subjects had symptomatic HIV-1 infection (i.e. stages II to IV), viz: 4 subjects (10%) instage II; 9 subjects (22.5%) in stage III and 17 subjects (42.5%) in stage IV. Out of these 34 subjects above the stage I classification, 23 subjects (57.5%) were already on therapy while the remaining 17 (42.5%) were yet to be put on therapy. Of the group that commenced treatment, 5 subjects (21.7%) were treated with only anti-retroviral drug (tablets) containing TDF/3TC/EFV at a dosage of 300mg/300mg/600mg respectively per day, while 18 subjects (78.3%) received 960mg per day tablets of Septrin (*Cotrimoxazole*) in addition to the above-mentioned ARV drug.

Trimethoprim or Atovaquone was given to those who reacted to Septrin. The paediatrics received lower doses of the ART compared to adults. These also include a fixed dose or triple fixed dose of AZT/3TC/NVP at dosage of 60mg/30mg/50mg and 300mg/150mg/200mg respectively. The subjects who were put on therapy had been on their respective medications for a period of 2± 1month before the follow-up blood samples were collected.

N/B: [3TC – Lamivudine, AZT – Zidovudine, NVP – Nevirapine, TDF – TenovirDisoproxil Fumarate, EFV – Efavirenz].

Table 1:
Comparison of Changes in Levels of Immunological Markers and Some Body Anthropometry in the Treatment and Non-treatment groups at Baseline and Follow-up. (Sample size, N= 40)

PARAMETERS	T test for equality of Means			
	Treatment group (Mean ± SD)	Non-treatment Group (Mean ± SD)	t-value	P-value
CD4 baseline	234.35 ± 185.26	454.94 ± 247.69	-3.226	0.003
CD4 follow-up	262.05 ± 225.83	421.94 ± 176.73	-2.385	0.022
Neopterin baseline	42.44 ± 40.68	54.24 ± 37.60	-0.936	0.355
Neopterin follow-up	30.54 ±17.50	32.29 ± 26.38	-0.252	0.802
B2M baseline	10581.88 ± 12963.28	11628.70 ± 13169.63	-0.251	0.803
B2M follow-up	14815.31 ± 17374.29	10156.51 ± 12703.88	0.935	0.356
Weight baseline	49.02 ± 16.76	61.82 ± 22.52	-2.063	0.046
Weight follow-up	50.89 ± 15.34	61.79 ± 22.62	-1.818	0.077
BMI baseline	18.99 ± 4.76	25.16 ± 6.72	-3.403	0.002
BMI follow-up	19.86 ±3.82	25.17 ± 6.74	-3.156	0.003

Statistically significant differences observed atp < 0.05

Table 2:
a. Immunological Profile of STAGE 1 HIV Subjects at Baseline and Follow-up. [Sample size, N= 10]

ID	Sex	Age	Baseline CD4 Count (cells/mm^3)	Follow-up CD4 Count (cells/mm^3)	Baseline NEO (nmol/L)	Follow-up NEO (nmol/L)	Baseline B2M (ng/mL)	Follow-up B2M (ng/mL)	Therapy (ARV)	Change in CD 4 Count
S1A	F	7	682±3	409±63	19.5±4.3	20.0±5.4	3880±2	8720±0.1	No	273±60 ↓
S1B	F	7	902±72	850±77	23.2±3.2	10.0±8.5	2730±3	4559±1	No	52±4 ↓
S1C	F	31	604±22	544±20	50.7±5.6	15.1±6.9	48182±12	3384±2	No	60±2 ↓
S1D	F	45	542±42	636±9	22.8±3.3	56.6±6.2	8416±1	20092±4	No	94±33 ↑
S1E	F	0.7	750±24	900±92	15.3±5.7	39.3±0.7	2150±3	8785±0	Yes	150±68 ↑
S1F	F	27	550±39	400±66	32.0±0.4	28.0±2.9	10901±0	5849±1	Yes	150±27 ↓
S1G	F	37	754±26	437±54	46.2±4.1	90.4±16.9	2430±3	6747±1	No	317±29↓
S1H	F	6	600±73	775±53	11.5±6.9	11.2±8.2	3560±2	12846±1	Yes	175±21 ↑
S1I	F	32	500±55	580±9	14.7±5.9	68.7±10.0	2060±3	14783±2	No	80±46↑
S1J	M	32	850±56	550±18	95.9±19.8	30.9±1.9	20830±3	2959±2	No	300±38 ↓
Total	**10**		**6734±412**	**6081±461**	**331.8±59.2**	**370.2±67.6**	**105139±32**	**88724±14.1**		**1651±328**

↑ *Increase in level or concentration;* ↓ *Decrease in level or concentration*

b: Statistical analysis of STAGE 1 HIV Subjects

	Pre	post	Z	p
CD4	673.40±136.89	608.10±180.39	1.096	0.302
NEO	33.18±25.68 *23.0 (15.2-47.3)*	37.02±26.93 *29.5 (14.1-59.6)*	-0.255	0.799
B2M	10513.9±14489.8 *3720.0 (2360.0-13383.3)*	8872.4±5517.5 *7733.5 (4265.3-13330.3)*	-0.459	0.646

Table 3
Immunological Profile of STAGE 2 HIV Subjects at Baseline and Follow-up after 3 months. [N= 4]

ID	Sex	Age	Baseline CD4 Count (cells/mm^3)	Follow-up CD4 (cells/mm^3)	Baseline NEO (nmol/L)	Follow-up NEO (nmol/L)	Baseline B2M (ng/Ml)	Follow-up B2M (ng/Ml)	Therapy	Change in CD 4 Count
S2A	F	27	400±18	452±25	137±30.1	10.9±12.7	30990±9	2411±8	No	52±7.5 ↑
S2B	F	46	482±23	284±59	68.4±4.2	19.3±8.4	5668±3	2434±7.7	No	198 ±35.5 ↓
S2C	F	34	458±11	420±9	22.2±27.3	86.7±25.3	5831±3	54092±18.1	No	38±2.0 ↓
S2D	F	35	402±17	450±24	79.6±1.4	27.7±4.2	5839±3	12332±2.7	No	48±7.5 ↑
TOTAL	**4**		**1742±69**	**1606±117**	**307.2±63**	**144.6±50.6**	**48328±18**	**71267±36.5**		**336±50.5**

Table 3b:
Showing the Statistical analysis of STAGE II HIV Subjects

	Pre	post	Z	p
CD4	435.50±41.03	401.50±79.69	0.581	0.602
NEO	76.80±47.20 *74.0 (33.8-122.7)*	36.15±34.39 *23.5 (13.0-72.0)*	-0.730	0.465
B2M	12082.0±12605.6 *5835.0 (5708.8-24702.3)*	17817.3±24630.2 *7383.0 (2416.8-43652.0)*	-0.365	0.715

With the assessment of the difference in concentration of each marker/parameter at baseline and follow-up using the paired T- test, only neopterin, BMI and waist/hip circumference had statistically significantly difference in concentration at both periods ($p < 0.05$). On the other hand, CD4 count and B2M concentration at baseline and follow-up had no significant difference ($p = 0.975$ and 0.596 respectively).

Moreover, with the adjustment of subjects' data by grouping them into those on therapy and those not on therapy, the independent sample T-test was used to assess the level of difference in concentrations of each parameter between the group on therapy and the non-therapy group at baseline and follow-up as shown in Table 2. Statistically significant differences were observed in CD4 count, waist/hip circumference and BMI between the group on therapy and those not on therapy ($p < 0.05$), whereas no statistically significant differences were seen between the concentrations of B2M and neopterin in the therapy versus non-therapy group.

In the determination of intra-parameter differences between the concentrations of baseline and follow-up in both therapy and non-therapy group using the 2-tailed paired sample T- test at 95% confidence internal (CI), as shown below in Table 3 and 4, no statistically significant difference was found between the baseline and follow-up levels of B2M and Neopterinin both therapy and non-therapy group. However, there were statistically significant differences in CD4 counts in both groups. Also, there were statistically significant differences between the baseline and follow-up body mass index (BMI) and weight in both therapy and non-therapy group.

Table 4

a. Immunological Profile of STAGE 3 HIV Subjects at Baseline and Follow-up after 3 months. [N= 9]

ID	Sex	Age	Baseline CD4 Count (cells/mm^3)	Follow-up CD4 (cells/mm^3)	Baseline NEO (nmol/L)	Follow-up NEO (nmol/L)	Baseline B2M (ng/mL)	Follow-up B2M (ng/mL)	Therapy	Change in CD 4 Count
S3A	F	50	341±21.1	400±35.6	18.3±13.9	16.6±3.9	19951±2.8	9699±0.8	Yes	59±14.56 ↑
S3B	F	35	206±23.9	195±32.7	185.7±41.9	25.6±0.9	21270±3.2	11280±0.3	Yes	11±8.77 ↓
S3C	F	36	347±23.1	400±35.6	13.6±15.5	14.6±4.5	3426±2.7	3719±2.8	No	53±12.56 ↑
S3D	F	38	200±25.9	185±36.0	70.5±3.5	26.7±0.5	30317±6.3	17982±2.0	No	15±10.11 ↓
S3E	M	28	340±20.7	Clotted	24.4±11.9	19.0±3.1	4273±2.4	3367±2.9	Yes	------
S3F	M	46	277±0.3	275±6.0	72.8±4.27	40.1±4.0	8963±0.9	13427±0.4	Yes	2±5.78 ↓
S3G	F	52	220±19.3	101±64.0	42.1±6.0	25.4±0.9	2767±2.9	7866±1.42	Yes	119±44.78 ↓
S3H	F	50	278±0.6	406±37.6	99.0±13.0	58.8±10.2	10345±0.4	21203±3.0	Yes	128±37.04 ↑
S3I	F	42	291±4.4	336±14.3	13.3±15.6	27.1±0.4	2793±2.9	20620±2.8	Yes	45±9.89 ↑
Total	**9**		**2223±139.3**	**2298±255.8**	**539.7±125.57**	**253.9±28.4**	**104105±24.5**	**109163±16.42**		**432±143.49**

b. Statistical analysis of STAGE III HIV Subjects

	Pre	post	Z	p
CD4	277.78±58.50 *278.0 (213.0-340.5)*	255.33±145.37 *275.0 (143.0-400.0)*	-0.059	0.953
NEO	59.97±56.19 *42.1 (16.0-85.9)*	28.21±13.69 *25.6 (17.8-33.6)*	-2.073	0.038*
B2M	11567.2±9988.6 *8963 (3109.5-20610.5)*	12129.2±6733.1 *11280.0 (5792.5-19301.0)*	-0.178	0.859

Tables 5:

a. Immunological Profile of STAGE 4 HIV Subjects at Baseline and Follow-up after 3 months. [N= 17]

ID	Sex	Age	Baseline CD4 Count (cells/mm^3)	Follow-up CD4 (cells/mm^3)	Baseline NEO (nmol/L)	Follow-up NEO (nmol/L)	Baseline B2M (ng/mL)	Follow-up B2M (ng/mL)	Therapy	Change in CD 4 Count
S4A	F	30	172±11.1	165±0.4	45.8±0.8	14.4±3.4	2615±0.7	4015±2.5	Yes	7±10.66 ↓
S4B	M	34	176±12.0	201±9.2	14.2±6.8	20.5±1.9	1729±0.9	6258±2.0	Yes	25±2.89 ↑
S4C	F	27	197±17.1	227±15.5	16.4±6.3	15.2±3.2	1755±0.9	3746±2.6	No	30±1.69 ↑
S4D	F	45	198±17.4	228±15.7	41.5±0.2	20.4±2.0	55701±12.2	4598±2.4	Yes	30±1.69 ↑
S4E	F	31	114±3.0	200±8.9	64.6±5.4	16.1±3.0	6529±0.2	2618±2.9	No	86±5.9 ↑
S4F	F	35	129±0.6	329±40.2	116.5±18.0	13.8±3.5	8682±0.8	9930±1.1	No	200±39.55 ↑
S4G	F	28	97±7.1	120±10.5	79.9±9.1	37.4±2.2	7297±0.4	8202±1.5	Yes	23±3.38 ↑
S4H	F	31	25±24.6	50±27.5	21.0±5.2	53.7±6.1	4916±0.2	3061±2.7	Yes	25±2.9 ↑
S4I	F	34	110±4.0	100±15.4	18.3±5.8	17.9±2.5	2583±0.7	3872±2.6	Yes	10±11.38 ↓
S4J	F	50	10±28.2	30±32.3	85.0±10.4	17.5±2.6	11093±1.4	11324±0.7	Yes	20±4.11 ↑
S4K	F	62	35±22.2	38±30.4	25.2±4.1	43.6±3.7	14040±2.1	16306±0.5	Yes	3±8.21 ↑
S4L	F	34	171±10.8	220±13.8	60.2±4.3	26.5±0.5	10124±1.1	2152±2.96	No	49±2.92 ↑
S4M	F	70	140±3.3	131±7.8	23.8±1.4	17.8±2.6	7288±5.3	28941±3.5	Yes	9±4.52 ↓
S4N	F	26	140±3.3	222±14.2	23.1±4.7	26.2±0.5	38018±3.6	23079±2.1	Yes	82±10.93 ↑
S4O	F	53	85±10.0	Clotted	59.4±4.2	17.0±2.8	4857±0.2	14226±0	Yes	------
S4P	F	41	150±5.7	110±12.9	13.6±7.0	85.4±13.8	4928±0.2	13988±0.1	Yes	40±7.18 ↓
S4Q	F	38	199±17.6	320±38.0	11.1±7.6	39.4±2.7	1347±1.0	87937±17.8	Yes	121±20.39 ↑
TOTAL	**17**		**2148±198**	**2691±292.7**	**719.6±101.3**	**482.8±57**	**183502±31.9**	**244253±47.96**		**760±138.3**

b. Statistical analysis of STAGE IV HIV Subjects

	Pre	post	Z	p
CD4	126.35±60.22 *140.0 (91.0-174.0)*	158.29±97.47 *165.0 (75.0-224.5)*	-2.012	0.044*
NEO	42.33±30.78 *25.2 (17.4-62.4)*	28.40±18.81 *20.4 (16.6-38.4)*	-1.349	0.177
B2M	10794.2±14378.1 *6529.0 (2599.0-10608.5)*	14367.8±20429.9 *8202.0 (3809.0-15266.0)*	-0.970	0.332

In order to determine the intra-parameter correlation or strength of relationship between the baseline and follow-up variables using the 2-tailed Pearson test of correlationas shown in table 5 below, a weak negative correlation was observed between the baseline and follow-up concentrations of beta 2 microglobulin (r = -0.122), as well as the baseline and follow-up concentrations of neopterin (-0.160). Hence, there was no statistically significant strength of relationship or correlation between the baseline and follow-up variables of these two markers ($p > 0.05$). In contrast, there was strong positive correlation between the pre and post values of other parameters including CD4 count, waist/hip circumferences, BMI, weight and height; which meant a that the later parameters had statistically significant strength of relationship or correlation between their baseline and follow-up variables ($p = 0.000$).

Furthermore, for the test of inter-parameter correlation using Pearson correlation, baseline and follow-up concentrations of both neopterin and B2M correlated significantly ($p < 0.05$) at baseline, $r_b = 0.293$, and follow-up, $r_f = 0.343$ respectively, but they did not correlate with CD4 count ($p > 0.05$).

In addition, CD4 count and weight were observed to correlate significantly with Age. However, there was no correlation between the mean income of the subjects and any of the HIV markers/parameters being assessed.

DISCUSSION

It has been established that the hallmark of HIV infection is the progressive reduction in CD4 T cell count and conversely, the increase in the concentrations of Neopterin and beta 2-microglobulin levels which is a reflection of immunological activation (Shi et al., 1997). The observation of a numerical decline in mean CD4+ T cells population as observed in Table 1 above (i.e. 334.18 to 333.58 cells/mm^3) may be attributed to T cell death caused by the HIV virus. The implication of this is the vulnerability of HIV seropositive individuals to various kinds of diseases/infections caused by their immunodeficient state (Ifeanyichukwu et al., 2011).

In the management of HIV infection, an increase in CD4 T cell counts is in most cases attributed to improved immune status, while a decrease in CD4 counts is in most cases seen as an indication of a failing immune system. As observed in this study, the follow-up levels of CD4 in the subjects on therapy (i.e.262.05 ± 225.83 cells/mm^3) were higher than the baseline level (i.e.234.35 ± 185.26cells/mm^3). This was an indication of improvement in immunity as well as a positive response to treatment. This finding is consistent with previous reports of increased CD4 level with interventions (i.e. nutrition and drugs) and good health practices (Simoni et al. 2011; Anochie et al. 2014). However, the intervention in this case centred more on the anti-retroviral (ARV) drugs that the therapy group received during the course of the study as a result of depleted CD4 count (i.e. ≤ 250 cells/mm^3).

The stimulation of human monocytes/macrophages by the cytokine called interferon-γ or lipopolysaccharide to produce neopterin has been well established (Wilmer et al. 1995; Murr et al., 2002). Consequently, the measurement of neopterin concentrations in body fluids provides information about the activation of immune response involving Type 1 T helper cells as a result of interaction between the human immunodeficiency virus (HIV) and the immune system (Hoffmann 1993; Schroecksnadel et al. 2004). The concentration of neopterin in healthy adults between 19-75yrs has been put at 5.3 ± 2.7nM (Murr et al. 2002 ;Schroecksnadel et al. 2004). As observed in this study, a higher concentration of neopterin at baseline (i.e. 47.45 ± 39.35nmol/L) was observed in the HIV positive subjects when compared with the follow-up concentration after 3 months (31.29 ± 21.42nmol/L) and the reference range concentration reported above from a another study. The dynamics in neopterin activity as observed in this study has been reported by previous studies i.e. the correlation of neopterin concentration with the presence of infections or disease in the body, including HIV (Fuchs et al. 1989;Anton et al. 1993). In HIV infection, neopterin has been observed to progressively increase in concentration up to the end of the viral incubation period, resulting in the detection of high neopterin levels even with the onset of clinical symptoms of HIV/AIDS (Murr et al., 2002). However, the level of neopterin is seen to drop as specific antibodies against the virus become detectable or at the commencement of HIV interventions such as ART (Murr et al. 2002; Schroecksnadel et al. 2004). This phenomenon probably explains the reason for the reduction of neopterin concentration (as well as B2M concentration) in both therapy and non-therapy group at follow-up. Moreover, the non-therapy group was seen to possess the highest level of neopterin at baseline as well as at follow-up.

The subjects that were studied included four (4) children who were between the ages of 3months to 7yrs (mean= 3.5yrs). It was observed that their values in the three immunological markers under study were not quite different from what was obtained in some adult subjects.

There was an observed decrease in the concentration of neopterin, following ART intervention, (i.e. 42.44nmol/L to 30.54nmol/L), as reported in other studies (Fuch et al. 1989 ;Peto 1996 ; Mildvan et al. 2005; Chandra et al.2005). The amount of neopterin production is of prognostic value, as documented by Murr et al., 2002. In order words, the decline in neopterin concentration is indicative of an improving immune status, whereas increases in neopterin concentration depicted disease progression.

Moreover, after seroconversion, neopterin concentrations are reported to decrease and normalise only when the body's immune system successfully combat the infecting agent. This is the situation in acute infections, whereas in chronic viral infections the level of neopterin is reported to decrease but not normalise (Murr et al., 2002). Hence neopterin is seen to have an inverse relationship with CD4 count, just as reported in other journals (Chandra et al. 2005; Gupta & Gupta, 2004)

HIV negative individuals have normal range of Beta 2-M concentrations between 0.9-2.4 mg/L in whites (Boffa, 1994) and 0.8-3.6 µg/ml in Nigerian subjects (Adedeji et al., 2012).These values are lower when compared with the mean concentration in HIV positive subjects in this study. Review of past literature supports the above stated observation (Moodley et al. 1996; Mocroft et al. 1997). Just as reported by Uppal et al., 2003, the pattern of changes in the concentrations of B2M was also observed to be inversely related to the pattern

of changes in the CD4 counts of the HIV subjects as seen in Tables 2a, 3a, 4a and 5a above. The mean baseline concentration of B2M (11026.78ng/mL) was seen to be higher than the follow-up concentration (12835.32ng/mL). This finding is also consistent with previous reports that documented an increase in the concentration of B2M with reductions in the levels of CD4 count (Coberly et al. 1992; Mocroft et al.1996).

Just as described for neopterin, the intake of ART had reducing effect on the concentration of B2M (Carstens et al., 1995). However, a comparison of B2M concentrations between the therapy and the non-therapy group at follow-up in this study revealed a higher concentration of B2M in the therapy group than the non-therapy (i.e. 14815.31ng/mL: 10156.51ng/mL respectively), but with the adjustment of B2M concentration based on sample size in the therapy group, there was found to be more reduction in the concentration of B2M in treatment compared to non-treatment, just as obtained in the studies cited above.

Although high levels of neopterin and beta 2-microglobulin was associated with low CD4 count, it was observed that levels of CD4 count had no significant relationship with neopterin and beta 2-microglobulin concentrations ($p > 0.05$) as shown in Tables 2b, 3b, 4b and 5b. This is consistent with studies that have reported the absence of association and relationship between CD4 count and B2M/Neopterin (Dolan et al. 1993; Shi et al. 1997). Moreover, there was observed a significant association between ART intake and CD4 counts ($p < 0.05$). In addition, it was observed that there was significant relationship ($p = 0.000$) and association ($p = 0.024$) between the level of CD4 count and HIV staging.

A statistically significant Increase or decrease was observed between baseline B2M and baseline neopterin; as well as follow-up B2M and follow-up neopterin ($p \leq 0.05$). This is consistent with Zeller et al. 1996; Dunne et al. 1996) .Furthermore, B2M and neopterin could not clearly predict severity of infection or mortality of infected subjects as observed with the baseline concentration of the two subjects who died in the course of the study with baseline concentrations of 3179.88ng/mL & 66844.38ng/mL for B2M, and 12.484nmol/L & 60.37nmol/L for neopterin. Although the second concentrations (i.e. 66844.38ng/mL and 60.37nmol/L) for B2M and neopterin respectively were reasonably high, similar or higher concentrations of both markers was seen in some other surviving subjects. However, a comparison of the ages of the dead subjects (i.e. 50yrs and 63yrs) to their surviving counterpart could be the possible distinguishing factor for the difference in the mortality rate between the subjects concerned. Moreover, this analogy would require a large sample based study to validate or truly give a reliable representation of the actual basis or predisposing factors of mortality in HIV patients with high B2M and neopterin concentration.

In conclusion, the study shows that B2M and neopterin are good immunologic markers but may not be adequate or reliable in monitoring short term progression of HIV/AIDS i.e. ≤3 months. However, further studies are required to ascertain their role on a long term basis (i.e. 6months to ≥1yr)

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