Neopterin and Biochemical parameters as indicators of predicting HIV disease progression and treatment response: A cross-sectional study in Ghana

ABSTRACT

Background: Surrogate markers have been identified to play significant role in the pathogenesis and prognosis of HIV infection. However, there is limited data on the utility of neopterin estimation in HIV infection. Therefore, the study sought to measure and ascertains the trends of serum neopterin and other biochemical parameters as indicators of predicting HIV disease progression and treatment response among HIV seropositive individuals.

Methods: A cross-sectional study with 298 HIV seropositive individuals consisting of 165 HIV on highly active antiretroviral treatment and 136 naïve highly active antiretroviral patients. Venous blood was drawn for the assay of neopterin and the other biochemical parameters.

Results: Neopterin was significantly lower (P<0.0001) in patients in the highly active antiretroviral therapy than those in the naïve highly active antiretroviral therapy group. Serum neopterin increased as the disease progresses and decreased as the duration of the therapy treatment increased (p=0.0001). At a cut of point of 54.5nmol/L, neopterin gave a sensitivity of 97.5%, specificity of 95.9% and an area under the curve of 0.99.

Conclusion: Neopterin has shown to be to be good marker in predicting HIV disease progression especially in patients with CD4 counts less than 200mm^{-3} and a useful indicator of patient's response to therapy treatment.

Keywords: Neopterin, Human Immunodeficiency Virus (HIV), Highly Active Antiretroviral therapy (HAART), CD4 counts

INTRODUCTION

HIV infection has been a challenge to the medical fraternity in the last three decades [1]. The use of HAART in the treatment of HIV infection has also resulted in a number of adverse effects [2, 3], hence the necessity of clinical assessment of the impact of HAART in the management of people living with HIV infection.

Several studies have shown the value of prognostic markers such as CD4 counts, viral RNA loads and soluble markers of immune activation in predicting HIV disease progression [4, 5] but the principal biomarker used in the monitoring of HIV infected individuals is the CD4 count. However, CD4 counts estimation is relatively expensive and require considerable skills as compared to other soluble biomarkers. In resource-limited settings, clinical monitoring of patients are difficult owing to the huge expenses associated with CD4 count test. Considering the fact that HIV infection is mostly endemic in ~~poor~~ developing/third world countries where resources and infrastructures are limited, there is the need for cost effective, easily performed and readily available surrogate markers that can assist in predicting the disease progression and patient's response to HAART.

A major feature of HIV infection is the activation of all component of the immune system and an increased production of several surrogate cytokines, which are indications of immunologic changes in the body during HIV infection. However, the limitation in the ability to measure circulating cytokines has led to the

determination of products of immune activation which reflect cytokine activity [9]. Such assessment includes soluble biomarkers and one such candidate marker is neopterin. Neopterin is a metabolite of guanosine triphosphate produced by macrophages and dendritic cells upon activation of gamma interferon. It is considered as a marker of immune activation and correlate with the disease progression [7, 10].

As neopterin levels reflect the degree of immune deficiency in HIV-positive patients, and perhaps the response to ART, the question of whether neopterin has (as claimed elsewhere) [11, 12], prognostic value concerning the HIV disease progression is eminent. It was against this premise that we evaluated serum neopterin and other biochemical parameters among HIV seropositive individuals on HAART and HAART naïve groups in other to ascertain the trends of these markers in HIV disease progression.

MATERIALS AND METHODS

STUDY DESIGN

This was a cross sectional study carried out in the ART clinic of the Bomso Specialist hospital in the Ashanti region of Ghana from August 2015 to March 2016.

STUDY POPULATION

Non-probability sampling technique was used to recruit 298 confirmed HIV seropositive individuals consisting of 162 HIV HAART patients and 136 HIV HAART naïve patients. Patients who were confirmed HIV seropositive and were 18 and above were recruited into the study. Patients with co-infection such hepatitis B, C, tuberculosis and pregnant women were excluded from the study. All participants were placed into three groups according to the center for disease control classification based on–CD4 lymphocytes of patients. The groups were; CD4 counts less than 200mm^{-3}, CD4 count between 200 and 499mm^{-3} and the third group consisted of patients with CD4 above 500mm^{-3}.

DATA COLLECTION AND LABORATORY ANALYSIS

A well-structured questionnaire was used to obtain demographic and clinical characteristics from the patients. 5ml of venous blood was taken from each patient under sterile conditions after a tourniquet has been applied for less than a minute. 2 ml out of the blood taken was place in an anti-coagulated sequestrene bottles-EDTA for CD4 and CD3 analysis using the Becton Dickenson and company haematological analyzer called the BD FACS Count from California in USA. The remaining blood was centrifuged after they have been made to clot in a plain test tube. The serum obtained was stored at -20^{o}C for the assay of neopterin (ELISA) and the other biochemical parameters using an auto-analyzer known as ATAC® 8000 Random Chemistry System from USA by Elan Diagnostic System.

DATA ANALYSIS

All the analysis were performed with the statistical package for social sciences version 20. The data were presented as median interquartile range (IQR) for non-parametric variables whiles grouped variables

were expressed as proportions. Comparison between HAART naïve and HAART patients were determined with the use of Mann Whitney U test. Spearman correlation rank test was used to determine the correlations between variables. A suitable cut off point was determined for neopterin using the Youden's index. The performance of neopterin was assessed using the area under the curve from the receiver operator characteristics. P-value < 0.05 was considered as statistically significant.

RESULTS

The socio-demographic and clinical characteristics of the studied population are shown in table 1. Out of the two hundred and ninety eight (298) participants, there were more females than male for both the HAART (70.2%) and the HAART naïve patients (69.3%). There was no statistically significance difference between the age of the HAART and HAART naïve group (p=0.203). The median CD4 counts of the HAART group (458 mm^{-3}) was significantly (p=0.0001) higher than the HAART naïve group (229 mm^{-3}). Although the median BMI was not statistically significant (p=0.521), the HAART group had a higher BMI (23.30 kg/m^2) compared to the HAART naïve group (22.55kg/m^2). None of the studied participants had a history of smoking and alcohol intake. There was no incidence of therapy discontinuation among the HAART group **[Table 1].**

Table 2 shows the comparison of biochemical parameters of the HAART and HAART naïve groups. Serum neopterin of the HAART naïve group (51.70nmol/L) was significant higher (p=0.0001) than the HAART group (26.40nmol/L). Serum albumin was significantly higher in the HAART group than the HAART naïve group (p=0.0001) whereas all the other biochemical parameters were significantly lower (p=0.0001) in the HAART group compared to the HAART naïve group.

Among the HAART group, patients on *Tenofovir, Lamivudine* and *Nevirapine* combination had the lowest mean serum levels of 27.7nmol/L whereas patients on *Zidovudine, Lamivudine* and *Nevirapine* combination had the highest mean serum neopterin levels of 45.nmol/L **[Figure 1].**

Table 3 shows the comparison of the biochemical parameters with the respective CD4 counts in the HAART and HAART naïve group. Neopterin increased as the disease progresses as measured by the respective CD4 counts in both the HAART and the HAART naïve groups. Elevated neopterin values were found in patients whose CD4 counts were below 200mm^{-3} whereas of neopterin lowest values were found in patients with CD4 counts ≥ 500mm^{-3} in both the HAART (17.20nmol/L) and the HAART naïve groups (21.40nmol/L).All the biochemical parameters except albumin also increased as the disease progresses.

Table 4 shows correlation of neopterin with CD4, biochemical parameters and some clinical factors**.** A significant negative correlation was observed between neopterin and CD4 count for both HAART (r= -0.99, p=0.0001) and HAART naïve patients (r= -0.96, p=0.0001). We observed a significant negative correlation between serum neopterin and albumin, BMI and the duration of therapy treatment for both the HAART and the HAART naïve groups. All the other biochemical parameters had a significant positive

correlation–with neopterin significantly except urea, which did not show any significant correlation with serum neopterin in the HAART naïve group.

Figure 2 shows the duration on HAART and serum neopterin levels among the HAART group**.** Neopterin levels decreased as the duration of HAART increased.

Table 5 shows the predictive performance of serum neopterin in predicting CD4 counts <200mm^{-3}. The diagnostic accuracy of serum neopterin in predicting CD4 less than 200mm^{-3} was found to be 83.9% . At cut off point of 55.4nmol/L, the sensitivity and specificity were found to be 97.5% and 95.9% respectively for the total participants. The area under the curve were also 0.99,1.0 and 0.98 for the total subject, HAART and HAART naïve groups repectively. From figure 2, neopterin levels were also found to be decreased as the duration of the therapy increased with patients being on the therapy for more than four years having the lowest serum neopterin levels (24.20nmol/L) followed by those who have been on the therapy between two and four years (28.90nmol/L). Highest neopterin values were found in patients who have been on the therapy for less than two years (32.80nmol/L).On the contrary CD4 count increased as the duration on the therapy increased.

Table 1: Socio-demographic and Clinical characteristics of the study participants

Parameter	HAART group(162)	HAART naïve(136)	P value

Age(years)	41 (35-53)	40 (31.3-50)	0.203
Gender			
Male	48(29.8%)	42(30.7%)	
Female	114(70.2%)	94(69.3%)	
CD4 (mm^{-3})	458.00(307.50-633.75)	229.00(136.25-338.75)	**0.0001**
CD3 (mm^{-3})	1216.50(931.00-1765.50)	919.00(667.50-1143.00)	**0.0001**
BMI kg/m^2)	23.30(20.33-26.85)	22.55(19.13-26.98)	0.521
HAART regime			
TDF+3TC+NVP	94(57.7)	---------	
AZT+3TC+ NVP	9(5.8)	---------	
CBV+NVP	28(17.3)	---------	
TDF+3TC+EFV	31(19.2)	---------	
HAART duration(yrs.)			
Median(IQR)	5(3-7)	--------	
Group1(<2)	10(5.2)	--------	
Group2 (2-4)	35(18.2)	--------	
Group 3(>4)	59(30.7)	--------	
Duration of diagnosis(yrs.)			
Median(IQR)	5(3-8)	1(0.45-2)	
Group1(<2)	6(3.1)	53(27.6)	
Group2 (2-4)	33(17.2)	31(16.1)	
Group 3(>4)	65(33.9)	4(2.1)	
Smoking	---------	--------	
Alcohol	---------	--------	
Drug discontinuation	---------	--------	

CD4- cluster of differentiation, IQR-interquartile range, CBV: Combivir, NVP: Nevirapine, EFV: Efavirenz, 3TC: Lamivudine, TDF: Tenofovir, AZV: Zidovudine.

Table 2: Comparison of biochemical parameters of the HAART group and HAART naïve group

Parameter	HAART group	HAART naïve	P value
Neopterin (nmol/L)	26.40(18.95-39.83)	51.75(35.60-67.70)	**0.0001**
Albumin (g/L)	40.05(36.50-41.28)	34.75 (32.10-39.10)	**0.0001**
Globulin (g/L)	57.00(48.95-74.98)	84.35 (69.83-92.03)	**0.0001**
Total Protein (g/L)	97.40(89.13-110.60)	118.90 (100.33-127.30)	**0.0001**
AST (U/L)	14.00(13.00-18.75)	23.00 (15.00-35.75)	**0.0001**
ALT (U/L)	10.00(8.00-14.00)	15.50 (10.00-25.00)	**0.0001**
Urea (mmol/L)	2.70(2.30-3.20)	3.20 (2.70-4.60)	**0.0001**
Creatinine (µmol/L)	62.00(55.00-69.75)	71.50 (62.00-84.50)	**0.0001**

AST-Aspartate amino Transferase, ALT-Alanine amino Transferase.

Table 3: Comparison of the biochemical parameters with the respective CD4 counts in the HAART and HAART naïve patients.

	CD4 Count		
	< 200	**200-499**	≥ 500
Parameters	**HAART naïve**		
Neopterin(nmol/L)	70.10(63.43-79.63)***	43.00(30.40-49.40) †††	21.40(19.55-23.55)###
Albumin(g/L)	32.10(30.33-33.20)***	36.40(34.30-39.30)††	40.20(39.70-40.35)###
Globulin(g/L)	88.05(83.63-97.03)***	81.40(60.10-90.10)††	55.10(49.90-58.50)###
Total protein(g/L)	119.70(116.60-127.40)	119.90(98.30-127.20)†	95.20(89.75- 98.75)###
AST(UI/L)	25.50(20.50-44.00)**	18.00(14.00-30.00)†	13.00(11.00-14.5)##
ALT(UI/L)	21.00(12.25-28.50)**	13.00(10.00-23.00)†	8.00(7.50-10.00)##
Urea(mmol/l)	3.60(2.65-5.10)	3.20(2.90-4.40)††	2.60(2.30-2.65)#
Creatinine(umol/l)	76.00(65.50-99.75)	68.00(62.00-82.00)††	55.00(53.50-60.00)##
	HAART group		
Neopterin(nmol/L)	70.10(60.13-75.50)***	32.60(27.45-41.15)†††	17.20(12.75-20.55)###
Albumin(g/L)	31.30(30.27-33.18)***	38.70(35.80-40.05)†††	41.20(40.25-42.45)###
Globulin(g/L)	86.05(75.73-89.90) **	64.80(57.75-80.55)†††	48.20(42.55-52.85)###
Total protein(g/L)	117.20(102.43-121.53)	97.40(100.70-119.90)†††	89.10(84.35- 94.15)###
AST(UI/L)	19.50(16.00-29.75)	17.00(14.00-20.00)†††	13.00(11.50-14.00)###
ALT(UI/L)	17.50(10.25-26.50)	12.00(10.00-14.00)†††	9.00(8.00-9.00)##
Urea(mmol/l)	2.95(2.28-5.13)	3.10(2.90-3.60)†††	2.3.00(2.20-2.50)#
Creatinine(umol/l)	69.00(57.00-85.25)	68.00(66.00-79.00)†††	55.00(53.00-57.50)###

*****P≤ 0.01, ***P≤ 0.0001 indicate level of significance when CD4 count < 200 was compared with CD4 200-499, †P≤ 0.05, ††P≤ 0.01, †††P≤ 0.0001 indicate level of significance when CD4 count 200-499 was compared with CD4 count ≥ 500, #P≤ 0.05, ##P≤ 0.01, ###P≤ 0.0001 indicate level of significance when CD4 count < 200 was compared with CD4 ≥500.***

Table 4 Correlation of Neopterin with CD4, biochemical parameters and some clinical factors.

Neopterin with	**HAART group**		**HAART naïve**		**CD4 with**	**HAART group**		**HAART naïve**	
	r	P value	r	P value		r	P value	R	P value
CD4	-0.995	0.0001	-0.964	0.0001					
Albumin	-0.786	0.0001	-0.674	0.0001	**Albumin**	0.797	0.0001	0.696	0.0001
Globulin	0.866	0.0001	0.426	0.0001	**Globulin**	-0.868	0.0001	-0.552	0.0001
Protein	0.802	0.0001	0.580	0.0001	**Protein**	-0.803	0.0001	-0.390	0.0001
AST	0.577	0.0001	0.507	0.0001	**AST**	-0.592	0.0001	-0.508	0.0001
ALT	0.506	0.0001	0.475	0.0001	**ALT**	-0.521	0.0001	-0.478	0.0001
Urea	0.741	0.0001	0.124	**0.250**	**Urea**	-0.749	0.0001	-0.132	**0.219**
Creatinine	0.766	0.0001	0.263	0.013	**Creatinine**	-0.764	0.0001	-0.254	0.017
BMI	-0.225	0.022	-0.516	0.0001	**BMI**	0.218	0.026	0.462	0.0001
DT	-0.289	0.003	-------	--------	**DT**	0.297	0.002	-------	--------

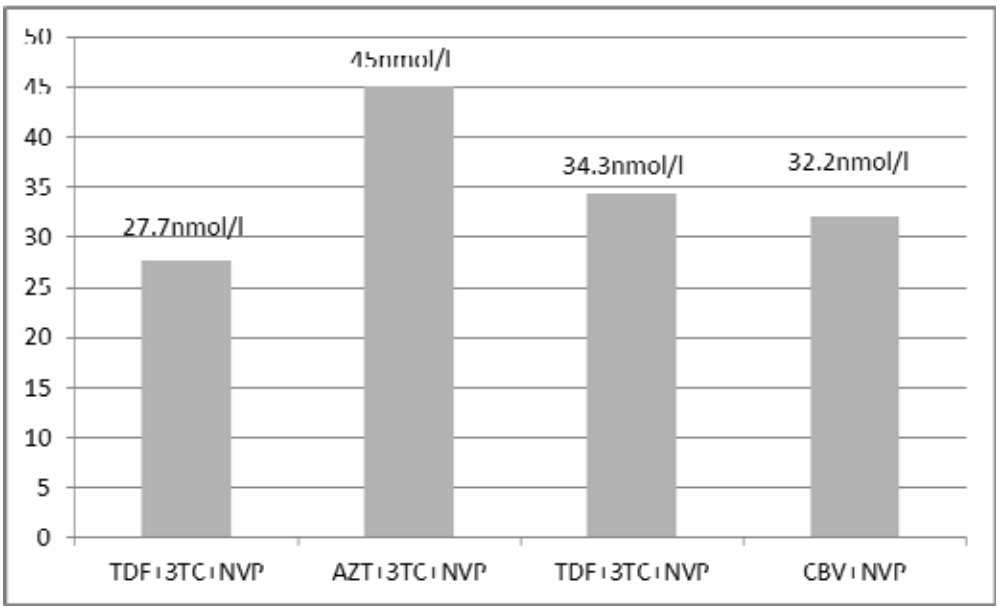
r: correlation coefficient, BMI-Body mass index, DT –duration of HAART treatment

Table 5: Predictive performance of serum neopterin in predicting CD4 counts <200mm^{-3}

	Cut off	Sen	Spec	PPV	NPV	AUC
Total subjects	55.4nmol/L	97.5%	95.9%	0.913	0.815	0.99
HAART group	58.0nmol/L	100%	100%	0.90	0.904	1.00
HAART naïve	59.5nmol/L	91.7%	100%	0.806	0.942	0.98

157 ***Sen; sensitivity, Spec; specificity, Accu; Accuracy, PPV; Positive predictive value, NPV; Negative***

158 ***predictive value, AUC; Area under the curve***



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160 **Figure 1** HAART types and their mean serum neopterin levels

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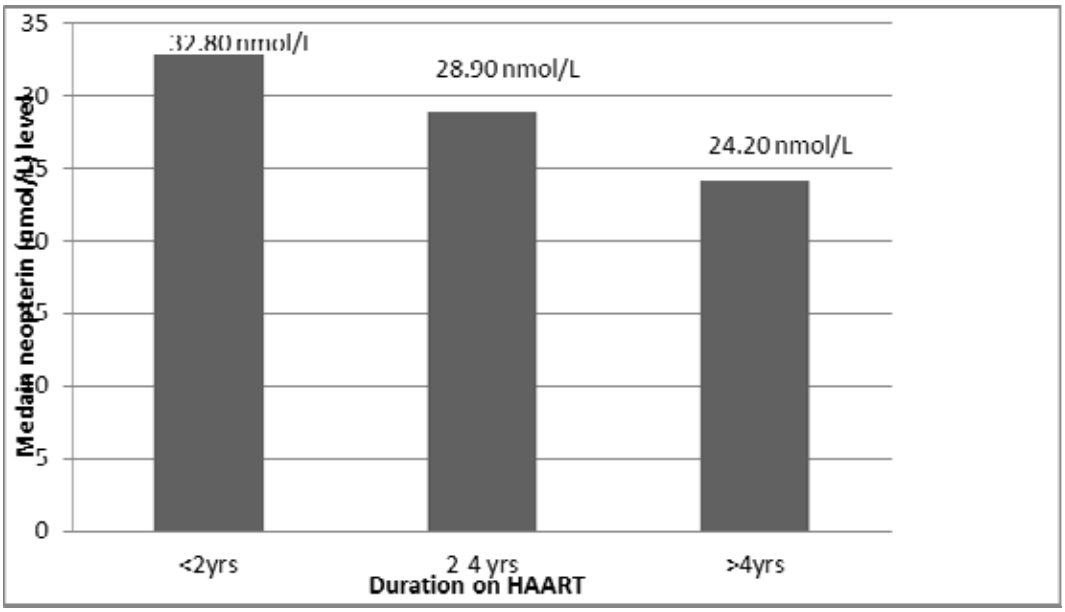

Figure 2: Duration on HAART and serum neopterin levels among the HAART patients.

DISCUSSION

Serum albumin levels in the HAART group was appreciably higher than the HAART naïve group (P≤0.0001). Albumin levels decreased as the disease progressed. These findings tie with earlier reports [13, 14] . Low albumin levels may be explained by poor nutritional status .Other possible mechanisms for decreased albumin levels may be attributed to the persistent inflammatory response caused by the infection. Also since the concentrations of acute phase protein are decreased in chronic inflammation as a result of elevation in cytokines level which imposes the liver to channel other proteins needed for immune responses, it was not surprising that albumin levels were decreased as the infection progressed given the fact that albumin is a negative acute phase protein. The serum globulin levels of the HAART naïve group were significantly higher than the HAART experienced group. Consistent with this study, other previous cross sectional studies that have evaluated serum globulins have found reduction in serum globulin in HAART groups compared to HAART naïve groups [15, 16]. Serum globulins increased as the disease progressed. This observation could be attributed to the chronic immune activation and B cell dysfunction which induces hypergamaglobulinemia via polyclonal B cell activation leading to a spontaneous increase in immunoglobulins which may result in the elevation of serum globulin in the HAART naïve group [17] Both AST and ALT of the HAART naïve group were higher than the HAART group. This is consistent with earlier findings by DNM Osakunor, C Obirikorang, V Fianu, I Asare and M Dakorah [18] and inconsistent with Ngala et al and Lucien et al. [2, 19].

There was no statistical difference in the body mass index of the HAART and HAART naïve groups (P=0.502). BMI was found to correlate positively with CD4 count for both the HAART and HAART naïve groups. Concurrent with this finding, previous study have reported higher BMI to be associated with increased CD4 counts as well as improved immune reconstitution and survival that could result~~ing~~ in a

slower disease progression [20-23]. The strong positive correlation between BMI and CD4 count is an indication that increased BMI may be associated with immunological improvement and a reduction in both immune activations that can contribute to a decrease in serum neopterin levels. In support of this, this study found serum neopterin to correlate negatively with CD4 count for both the HAART and the HAART naïve groups.

Serum neopterin levels were significantly lower in patients who were on the highly active antiretroviral therapy than the naïve highly active antiretroviral patients ($P<0.0001$). This result correlates well with studies by Bipath et al. [24] and N Amirayan-Chevillard, H Tissot-Dupont, Y Obadia, H Gallais, JL Mege and C Capo [25] who indicated that HAART significantly decrease circulating levels of neopterin by 30 % (61.7nmol/L and 88.1nmol/L for HAART and HAART naïve respectively). HIV infection is associated with a continuous immune activation which stimulates the release of inflammatory cytokines by increasing the total level of neopterin in the HAART naïve group. Several explanations could be attributed to the increased immune activation seen in HIV infection. At the site of HIV infection, there is migration of tissue peripheral CD4 cells which results in the activation of macrophages. This leads to a reduction in the peripheral blood CD4 cells which contributes to a persistent immune activation. Neopterin levels were found to be increased as the disease progressed with higher median values in patients with CD4 counts $<200\ mm^{-3}$. This result is in line with studies by S Chadha, P Bhalla, H Gautam, A Chakravarti, S Saini, S Anuradha and R Dewan [7] who also reported higher neopterin values in patients with CD4 counts less than $200mm^{-3}$. This elevation could be due to the stimulatory role of γ IFN in the synthesis of neopterin [26] and the link between chronic elevation of γ IFN and HIV disease progression [12]. D Mildvan, J Spritzler, SE Grossberg, JL Fahey, DM Johnston, BR Schock and J Kagan [10] also reported that elevated baseline neopterin levels are linked with increased risk of HIV disease progression as well as the predictive value of neopterin in the disease progression.

Among the HAART group, we observed a significant positive correlation between CD4 and duration on therapy (rho 0.297, p=0.002) whereas a negative significant correlation was observed between the duration on therapy and neopterin (rho -0.289, p=0.003). This is an indication that increased duration on HAART may result in an increase in CD4 and a decline in serum neopterin levels. This results is backed by earlier studies conducted by Bipath et al. [11]. Several mechanisms have been proposed for the effect of these therapies on serum neopterin levels of patients infected with HIV. In a randomized doubled blind study aimed at evaluating the effect of HAART treatment on the human immunodeficiency virus, AC Collier, RW Coombs, DA Schoenfeld, RL Bassett, J Timpone, A Baruch, M Jones, K Facey, C Whitacre, VJ McAuliffe, et al. [27] found an association between HAART and an increase in the CD4 T lymphocyte cells and a decline in markers of immune activation; which promotes a partial recovery in functions of the immune cells. This was consistent with the results that CD4 count increased among HAART group than the HAART naive group and a decrease in neopterin levels among the HAART group. In another cross sectional study, the functions of the lymphoid progenitor cells including the differentiation into the B and T

lymphocytes have also been shown to normalize among HIV patients treated with HAART [28]. HAART has been shown to induce changes in the T helper 1 and T helper 2 which differentiate to produce cytotoxic T cells and antibodies respectively [29] and also reverses the defects in the CD4 cells [30]. The cumulative effect of these mechanisms of HAART on the immune cells is the restoration of interleukin 2 productions that modulate various aspect of the immune response [31] and a decline in the level of cytokines involved in the pathogenesis of HIV infection. Consequently there is a reduction in the immune activation and the circulating levels of serum neopterin in HIV infected individuals [12]. Although HAART was associated with a decrease in serum neopterin levels, the usage of Tenofovir, Lamivudine and Nevirapine combination resulted in a much more decrease in serum neopterin levels compared with the other HAART combinations used in this study.

CONCLUSION

Neopterin levels were elevated in HAART naïve group compared to HAART group. Increased neopterin levels were associated with a decline in CD4 count and correlated with severity of the disease progression whereas longer duration on the therapy was associated with an increase in CD4 count and a decline in neopterin levels. The results of this study has revealed that neopterin can be a useful biomarker in predicting HIV disease progression especially in patients with CD4 counts less than 200mm^{-3} and a useful indicator of patient's response to therapy treatment. Regular measurement of serum neopterin and other biochemical parameters could provide some prognostic information on the disease progression to enhance close monitoring and therapeutic interventions for individuals with a greater possibility of progressing with the disease in resource limited settings.

CONSENT FOR PUBLICATION

Not Applicable

ETHICAL APPROVAL

Ethical approval was sought from the management of Bomso specialist Hospital and the committee on human research and publication of the School of medical science, Kwame Nkrumah University of Science and Technology (KNUST). Participation was voluntary and verbal informed consent was obtained from each participant according to Helsinki declaration. Respondents were assured that the information gathered was to be used strictly for research and academic purpose only. In addition, respondents were given the freedom to opt out any time they thought they couldn't continue with the study.

REFERENCE

1. UNAIDS: **HIV Epidemic Update. Geneva**. 2002.
2. Ngala RA, Opoku D, Asare G: **Effects of HIV Infection and Highly Active Antiretroviral Therapy (HAART) on the Liver of HIV Patients**. *Trends in Medical Research* 2015, **10**(1):1-11.
3. Ejilemele A, Nwauche C, Ejele O: **Pattern of abnormal liver enzymes in HIV patients presenting at a Nigerian Tertiary Hospital**. *The Nigerian postgraduate medical journal* 2007, **14**(4):306-309.
4. Fuchs D, Albert J, Åsjö B, Fenyö E, Reibnegger G, Wachter H: **Association between serum neopterin concentrations and in vitro replicative capacity of HIV-1 isolates**. *Journal of Infectious Diseases* 1989, **160**(4):724-725.
5. Fahey JL: **Cytokines, plasma immune activation markers, and clinically relevant surrogate markers in human immunodeficiency virus infection**. *Clinical and diagnostic laboratory immunology* 1998, **5**(5):597-603.
6. Balakrishnan P, Solomon S, Kumarasamy N, Mayer KH: **Low-cost monitoring of HIV infected individuals on highly active antiretroviral therapy (HAART) in developing countries**. *Indian Journal of Medical Research* 2005, **121**(4):345.
7. Chadha S, Bhalla P, Gautam H, Chakravarti A, Saini S, Anuradha S, Dewan R: **Utility of Serum Neopterin and Serum IL-2 Receptor Levels to Predict Absolute CD4 T Lymphocyte Count in HIV Infected Cases**. *Interdiscip Perspect Infect Dis* 2013, **2013**:143648.
8. Malone JL, Simms TE, Gray GC, Wagner KF, Burge JR, Burke DS: **Sources of variability in repeated T-helper lymphocyte counts from human immunodeficiency virus type 1-infected patients: total lymphocyte count fluctuations and diurnal cycle are important**. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 1990, **3**(2):144-151.
9. Chandra I, Lalitha V, Chelvi KS, Padmapriyadarsini C: **Serum neopterin levels in HIV infected patients with & without tuberculosis**. *Indian Journal of Medical Research* 2005, **121**(4):220.
10. Mildvan D, Spritzler J, Grossberg SE, Fahey JL, Johnston DM, Schock BR, Kagan J: **Serum neopterin, an immune activation marker, independently predicts disease progression in advanced HIV-1 infection**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2005, **40**(6):853-858.
11. Bipath P, Viljoen M, Levay PF: **Levels of procalcitonin, C-reactive protein and neopterin in patients with advanced HIV-1 infection**. *Southern African Journal of HIV Medicine* 2012, **13**(2):78-82.
12. Wirleitner B, Schroecksnadel K, Winkler C, Fuchs D: **Neopterin in HIV-1 infection**. *Mol Immunol* 2005, **42**(2):183-194.
13. Mehta SH, Astemborski J, Sterling TR, Thomas DL, Vlahov D: **Serum albumin as a prognostic indicator for HIV disease progression**. *AIDS Research & Human Retroviruses* 2006, **22**(1):14-21.
14. Olawumi HO, Olatunji PO: **The value of serum albumin in pretreatment assessment and monitoring of therapy in HIV/AIDS patients**. *HIV Med* 2006, **7**(6):351-355.
15. Jacobson MA, Khayam-Bashi H, Martin JN, Black D, Ng V: **Effect of long-term highly active antiretroviral therapy in restoring HIV-induced abnormal B-lymphocyte function**. *Journal of acquired immune deficiency syndromes* 2002, **31**(5):472-477.
16. Serpa J, Haque D, Valayam J, Breaux K, Rodriguez-Barradas MC: **Effect of combination antiretroviral treatment on total protein and calculated globulin levels among HIV-infected patients**. *International Journal of Infectious Diseases* 2010, **14**:e41-e44.
17. Moir S, Fauci AS: **B cells in HIV infection and disease**. *Nature reviews Immunology* 2009, **9**(4):235-245.
18. Osakunor DNM, Obirikorang C, Fianu V, Asare I, Dakorah M: **Hepatic Enzyme Alterations in HIV Patients on Antiretroviral Therapy: A Case-Control Study in a Hospital Setting in Ghana**. *PloS one* 2015, **10**(8):e0134449.

19. Lucien K, Clement A, Fon N, Weledji P, Ndikvu C: **The effects of antiretroviral treatment on liver function enzymes among HIV-infected out patients attending the Central Hospital of Yaounde, Cameroon**. *African Journal of Clinical and Experimental Microbiology* 2010, **11**(3).
20. Jones CY, Hogan JW, Snyder B, Klein RS, Rompalo A, Schuman P, Carpenter CC, Group HIVERS: **Overweight and human immunodeficiency virus (HIV) progression in women: associations HIV disease progression and changes in body mass index in women in the HIV epidemiology research study cohort**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2003, **37 Suppl 2**(Supplement 2):S69-80.
21. Koethe JR, Jenkins CA, Shepherd BE, Stinnette SE, Sterling TR: **An optimal body mass index range associated with improved immune reconstitution among HIV-infected adults initiating antiretroviral therapy**. *Clinical infectious diseases* 2011:cir606.
22. Denue B, Ikunaiye P, Denue C: **Body mass index changes during highly active antiretroviral therapy in Nigeria/Modifications de l'indice de masse corporelle pendant le traitement antirétroviral hautement actif au Nigéria**. *Eastern Mediterranean Health Journal* 2013, **19**(3):S89.
23. Kwantwi LB, Tunu BK, Boateng D, Quansah DY: **Body Mass Index, Haemoglobin, and Total Lymphocyte Count as a Surrogate for CD4 Count in Resource Limited Settings**. *Journal of biomarkers* 2017, **2017**.
24. Bipath P, Levay P, Olorunju S, Viljoen M: **A non-specific biomarker of disease activity in HIV/AIDS patients from resource-limited environments**. *African health sciences* 2015, **15**(2):334-343.
25. Amirayan-Chevillard N, Tissot-Dupont H, Obadia Y, Gallais H, Mege JL, Capo C: **Highly active antiretroviral therapy (HAART) and circulating markers of immune activation: specific effect of HAART on neopterin**. *Clin Diagn Lab Immunol* 2000, **7**(5):832-834.
26. Murr C WB, Wirleitner B, Fuchs D.: **Neopterin as a marker for immune system activation.** *Curr Drug Metab* 2002, **3**:175-187.
27. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, Jones M, Facey K, Whitacre C, McAuliffe VJ *et al*: **Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group**. *The New England journal of medicine* 1996, **334**(16):1011-1017.
28. Nielsen SD, Ersbøll AK, Mathiesen L, Nielsen JO, Hansen J-ES: **Highly active antiretroviral therapy normalizes the function of progenitor cells in human immunodeficiency virus-infected patients**. *Journal of Infectious Diseases* 1998, **178**(5):1299-1305.
29. Pakker NG, Notermans DW, de Boer RJ, Roos MT, de Wolf F, Hill A, Leonard JM, Danner SA, Miedema F, Schellekens PT: **Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation**. *Nat Med* 1998, **4**(2):208-214.
30. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, Katlama C, Debre P, Leibowitch J: **Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease**. *Science* 1997, **277**(5322):112-116.
31. Daniel V, Süsal C, Melk A, Weimer R, Kröpelin M, Zimmermann R, Huth-Kühne A, Uhle C, Opelz G: **Reduction of viral load and immune complex load on CD4+ lymphocytes as a consequence of highly active antiretroviral treatment (HAART) in HIV-infected hemophilia patients**. *Immunology letters* 1999, **69**(2):283-289.