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 as having significance in the pathogenesis and prognosis of HIV infection. But there is limited data on the utility of neopterin estimation in HIV infection. Therefore the study sought to measure and ascertains the trend 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 in 298 HIV seropositive individuals consisting of 165 HIV on highly active antiretroviral treatment and 136 naïve highly active antiretroviral patients. Venous blood was taken for the assay neopterin and the other biochemical parameters.

Results: Neopterin was significantly lower (p=0.0001) in patients placed on the highly active antiretroviral therapy than the naïve highly active antiretroviral therapy patients. Serum neopterin was found to increase as the disease progresses and decreases as duration of the therapy treatment increases (p=0.0001). At 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 the HIV disease progression especially in patients with CD4 counts less than 200mm^{-3} and 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 infected individuals has also resulted in a number of adverse effects [2, 3], hence the clinical assessment of the impact of HAART has become necessary 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 the disease progression [4, 5] but the principal biomarker marker 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 hence few laboratories in resource limited settings offer the test for patients making the clinical monitoring of patients difficult [6-8]. 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 the HIV infection. However, due to 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. Hence it 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 was asked whether neopterin has indeed, as claimed elsewhere [11, 12], prognostic value concerning the HIV disease progression. It was against this premise that we evaluated serum neopterin and other biochemical parameters among HIV seropositive on HAART and HAART naïve HIV patients 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 at the ART clinic of the Bomso Specialist hospital in the Ashanti region of Ghana from August 2015 to March 2016. Bomso Specialist hospital is staffed by registered general nurses, medical doctors, medical assistance, laboratory technicians, nutritionist, surgeons, health assistants, psychiatrist, optometrist and laborers. The department presently used by the hospital for it service provision are the outpatients department (OPD), in-patient department, maternity wing, laboratory unit, pharmacy department, a theater, a psychiatry unit, an x-ray unit and a mortuary

STUDY POPULATION

Non-probability sampling technique was used to recruit a total of 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 which indicates the 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 were 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°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

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 was carried out using Mann Whitney U test. Spearman correlation rank test was used to assess correlations between variables. A suitable cut off point were 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 less than 0.05 was statistically considered to be significant. All the analysis was performed using the statistical package for social sciences version 20.

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 statically 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 drinking. There was also no incidence of therapy discontinuation among the HAART group **[Table 1].**

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

Among the HAART group, patients on Tenofovir, Lamivudine and Nevirapine combination had the lowest mean serum levels 27.7nmol/L whiles patients on Zidovudine, Lamivudine and Nevirapine combination had the highest mea 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 patients. Neopterin was found to be increased as the disease progresses as measured by the respective CD4 counts in both the HAART and the HAART naïve patients. Elevated neopterin values were found in patients whose CD4 counts were below 200mm^{-3} whiles lowest values were found in patients with CD4 counts ≥ 500mm^{-3} in both the HAART (17.20nmol/L) and the HAART

naïve patients (21.40nmol/L).All the biochemical parameters except albumin were 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). Correlation of serum neopterin with albumin, BMI and the duration of therapy treatment also showed significant negative correlation for both the HAART and the HAART naïve subjects. All the other biochemical parameters correlated positively to neopterin significantly except urea which did not show any significant correlation with serum neopterin in the HAART naïve patients.

Figure 2 shows the duration on HAART and serum neopterin levels among the HAART patients**.** Neopteri levels decreases as the duration of HAART increases.

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 patients repectively. From figure 2, neopterin levels were also found to be decreased as the duration of the therapy increases with patients being on the therapy for more than four 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 was increased as the duration on the therapy increases.

Table 1: Socio-demographic and Clinical characteristics of the studied 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

Sen; sensitivity, Spec; specificity, Accu; Accuracy, PPV; Positive predictive value, NPV; Negative predictive value, AUC; Area under the curve

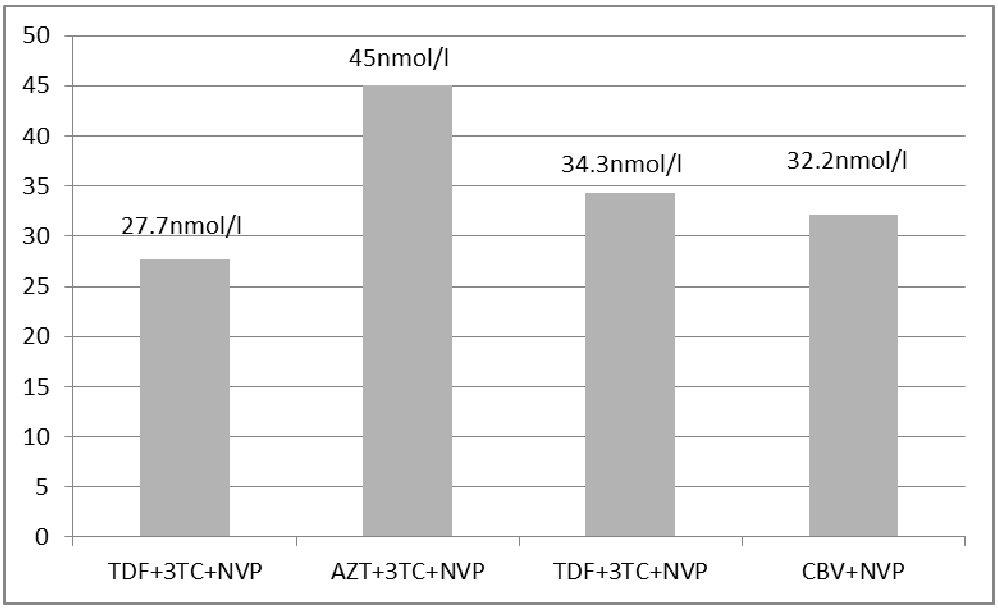

Figure 1 HAART types and their mean serum neopterin levels

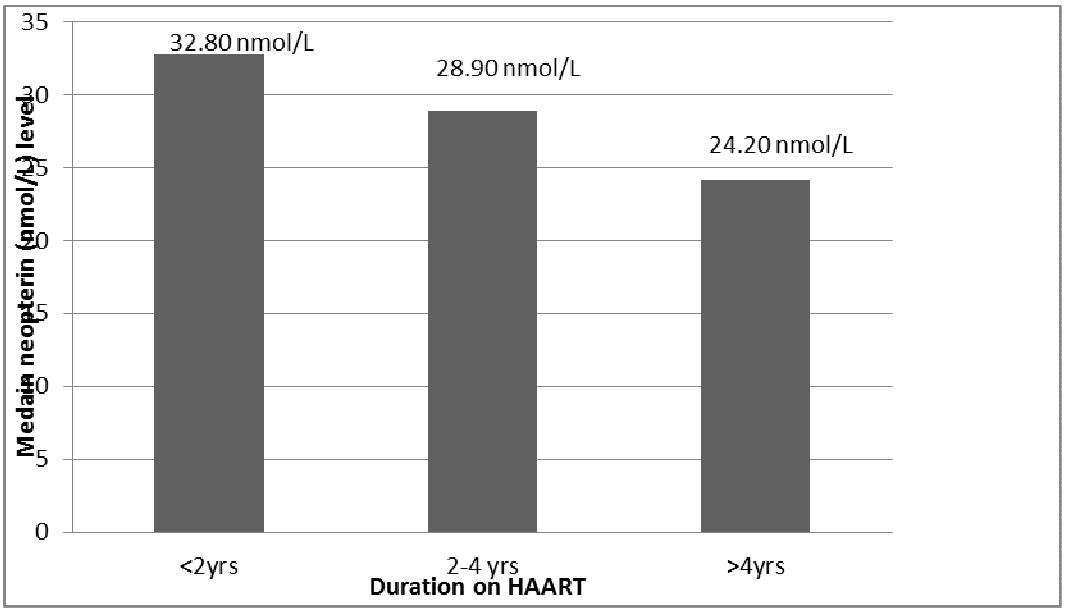

Figure 2: Duration on HAART and serum neopterin levels among the HAART patients.

DISCUSSION

Serum albumin levels in the HAART patients was appreciably higher than the HAART naïve patients (p=0.0001). Albumin levels were found to be decreased as the disease progresses. These findings tie with earlier reports by [13, 14] . Low albumin levels may be explained by poor nutritional status .Other possible mechanisms for decreased albumin may be due 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 response, it was not surprise that albumin levels were decreased as the infection progresses given the fact that albumin is a negative acute phase protein. The serum globulin levels of the HAART naïve patients were significantly higher than the HAART experienced patients. Consistent with this study, other previous cross sectional studies that have evaluated serum globulins have found reduction in serum globulin in HAART patients compared to HAART naïve patients [15, 16]. Serum globulins were found to be increased as the disease progresses. 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 patients [17] Both AST and ALT of the HAART naïve patients was higher than the HAART patients. This concur with earlier findings by Osakunor et al. [18] and inconsistent with [2, 19].

There was no statistical difference in the body mass index of the HAART and HAART naïve patients (p=0.502). BMI was found to correlate positively with CD4 count for both the HAART and HAART naïve patients. 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 resulting in a slower

disease progression [20-22]. The strong positive correlation between BMI and CD4 count is indicative that increased BMI may be associated with immunological improvement and a reduction in both immune activations contributing to a decrease in serum neopterin levels. In support of this, the study found serum neopterin to correlate negatively with CD4 count for both the HAART and the HAART naïve patients.

Serum neopterin levels was 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 [23] and N Amirayan-Chevillard, et al. [24] who showed 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 thereby 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 thereby contributing to a persistent immune activation. Neopterin levels were found to be increased as the disease progresses with higher median values in patients with CD4 counts <200 mm^{-3} and this collaborate with studies by Chadha et al [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 [25] and the link between chronic elevation of γ IFN and HIV disease progression [12]. Mildvan et al [10] also reported that elevated baseline neopterin levels are linked with increased risk of HIV disease progression and indicate the predictive value of neopterin in the disease progression.

Among the HAART patients, the study observed a significant positive correlation between CD4 and the duration on the therapy (rho 0.297, p=0.002) whiles a negative significant correlation was observed between the duration on the therapy and neopterin (rho -0.289, p=0.003).This is thus indicative that increased duration on HAART may result in an increase in CD4 and a decline in serum neopterin levels. This is consistent with earlier report by [11] Several mechanisms have been proposed as to the effect of these therapies on the 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, Collier et al. [26] found HAART to be associated with 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 which found CD4 count to be increased among HAART patients than the HAART naive subject and a decrease in neopterin levels among the HAART patients. 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 the highly active antiretroviral therapy [27]. 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 [28] and also reverses the defects in the CD4 cells [29]. The cumulative effect of these mechanisms of HAART on the immune cells

is the restoration of interleukin 2 productions which modulate various aspect of the immune response [30] 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 patients compared to HAART patients. Increased neopterin levels were associated with a decline in CD4 count and correlated with severity of the disease progression whiles longer duration on the therapy was associated with an increase in CD4 count and a decline in neopterin levels. Neopterin has shown to be a useful biomarkers in predicting the 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 enhancing 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.

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