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Haptoglobin phenotypes with weak antioxidant capacity increase risk factors of cardiovascular disease in Ghanaian HIV-infected patients on highly active antiretroviral therapy

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Abstract

OBJECTIVE Highly active antiretroviral therapy (HAART) has considerably reduced HIV/AIDS-related morbidity and mortality; however, the therapy has been associated with the development of cardiovascular disease (CVD), and genetic predisposition factors may aggravate disease outcome. This study was aimed at investigating the relationship between haptoglobin phenotypes and risk factors of CVD in HIV patients.

METHODS A total of 105 HIV sero-positive patients on HAART and 75 HIV-infected HAART-naïve individuals were enrolled in the study. Socio-demographics and clinical characteristics of the participants were obtained using a well-structured questionnaire. Lipid profile, lactate dehydrogenase (LDH) and haptoglobin (Hp) phenotypes were analysed from serum whiles haemoglobin (Hb) level, $CD4^+$ cell count and HIV viral RNA load were determined using whole blood.

RESULTS Atherogenic index of plasma (AIP) was significantly higher in patients on HAART than the naïve group ($P < 0.05$). Age, BMI, visceral fat, systolic blood pressure LDH and lipid variables strongly and positively correlated with AIP ($P < 0.05$), with the exception of HDL-c ($P < 0.001$) which showed a negative correlation. HAART was associated with hypertension ($\chi^2 = 4.33$, $P = 0.037$), hypercholesterolaemia ($\chi^2 = 10.99$, $P < 0.001$), elevated LDL-c ($\chi^2 = 10.30$, $P < 0.001$) and decreased HDL-c ($\chi^2 = 3.87$, $P = 0.09$). Hp2-2 and Hp0 collectively was strongly associated with hypertension (OR = 2.54, $P = 0.011$), obesity (OR = 5.97, $P < 0.001$) and hypercholesterolaemia (OR = 2.99, $P < 0.001$).

CONCLUSION HIV/AIDS patients on HAART expressing Hp phenotypes with weak antioxidant capacity have an increased risk of developing CVD.

keywords haptoglobin phenotype, HIV, highly active antiretroviral therapy, cardiovascular disease, antioxidant

Introduction

Despite several attempts to combat HIV/AIDS, the disease still remains a global threat, especially in low- and middle-income countries [1]. Recent data suggest that 36.9 million people are living with HIV/AIDS, with an estimated 2.4 million new infections and 1.3 million deaths due to HIV-related illnesses [2]. Introduction of the highly active antiretroviral therapy (HAART) has decreased HIV/AIDS burden considerably, with global access to HAART exceeding the United Nations Assembly target of 15 million patients by 2015 [3]. With HAART intervention, global annual HIV infection incidence dropped by 38% from 3.4 to 2.1 million people between 2001 and 2013 [4]. In South Africa, percentage of deaths related to HIV/AIDS declined steadily, and were attributed to the roll-out of antiretroviral therapy [5, 6]. Mother-to-child transmission in certain parts of the world has been eliminated due to increase in the number of HIV-infected pregnant women enrolled in

disease management programs [4]. Currently, the WHO recommended treatment plan has categorised HAART into first-, second- and third-line, and factors considered for the selection of patients include cardiovascular disease and hyperlipidaemia [7, 8]. The call to scale up HAART administration in the test-and-treat policy is expected to further decrease HIV-related mortality worldwide [9].

Notwithstanding the success in decreasing morbidity and mortality related to HIV infection, studies have associated HAART with increased risk of developing cardiovascular disease (CVD) [10, 11]. Even though the incidence of CVD in HIV patients is a major public health issue, the underlying cause of the increased risk factors in the patients is not clear. HIV infected patients on HAART have been reported to show high prevalence of obesity, hypertension, hypertriglyceridemia, high level of low-density lipoprotein cholesterol (LDL-c), reduced levels of high density lipoprotein cholesterol (HDL-c), high atherogenic index, visceral fat and treatment-induced oxidative stress compared to HAART-naïve individuals [12–15]. Assessing the risk factors of CVD in HIV patients on HAART will help in the selection of appropriate management plan to reduce treatment-associated morbidity.

Haptoglobin (Hp) is a glycoprotein with variable antioxidant capacity [16]. The physiological importance of Hp depends on the phenotypic presentation in individuals, with the most common phenotypes being Hp1-1, Hp2-1 and Hp2-2 [17]. Phenotype Hp1-1 shows strong antioxidant property followed by Hp2-1, while Hp2-2 remains the weakest [16]. Hp0 is the absence of Hp in blood, which has been attributed to mutation and/or deletion of the promoter region of the *HP* gene and excessive haemolysis, and results in null expression and rapid clearance of the circulatory protein, respectively [18, 19]. Different Hp phenotypes have been associated with different diseases due to disparities in free radical clearance by the respective phenotypes [20, 21]. Poor clearance of free haemoglobin (Hb) in circulation by Hp2-2 phenotype increases iron availability for growth of iron-dependent bacteria [22], and the phenotype has also been reported to increase mortality rate of HIV-infected patients compared to Hp1-1 and Hp2-1 [23]. Hp2-2 increased viral titres in HIV-infected patients via free iron-induced oxidative stress, and enhances the oxidation of LDL-c, a precursor of atherosclerotic CVD [23, 24]. Establishing Hp phenotype status will highlight the need for appropriate management alternatives using antioxidant supplements in HIV patients with weak antioxidant Hp phenotypes to reduce the incidence of CVD. Although studies have demonstrated the significance of the weak antioxidants of Hp phenotypes in HIV disease progression, information on the relation between the phenotypes and risk factors of CVD in patients on HAART has not been established. This study was designed to investigate the impact of Hp phenotypes on dyslipidemia and other risk factors of CVD in Ghanaians infected with HIV.

Materials and methods

Study site and study population

This study was carried out at the Fevers Unit of the Department of Medicine, Korle-Bu Teaching Hospital, Accra, Ghana; the leading national referral hospital in Ghana. This was a cross-sectional comparative study in which participants attending clinic at the Fevers Unit were randomly selected from July, 2015 to July, 2016. A total of 180 HIV-1-infected individuals, made up of 105 patients who have been on HAART for more than a year and 75 HAART-naïve, were recruited into the study. Patients diagnosed with other chronic diseases including cancers, chronic liver disease, diabetes, kidney disease, and cigarette smokers, excessive alcohol consumers and/or pregnant women were excluded. The study was approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana (CHS-Et/M4-P3.11/2015–2016). The study was explained to the participants and consent form was signed by those who agreed to participate in the study. Participants were made to understand that not participating in the study would not affect their healthcare at the unit.

Collection of demographic and clinical data of participants

The study adopted the data collection procedure as described by Nsagha *et al.* [10]. In summary, standard questionnaire was used to capture socio-demographic characteristics, and patients' treatment regimen was retrieved from medical records after permission has been given by the hospital authority. Anthropometric measurements, including height and weight were taken with metre rule and weighing scale, respectively. Height of the patients was taken by standing in an erect position on a flat floor without footware, and the weight was measured in light clothing. A digital body fat analyser (Omron, Model HBF-514) was used to measure total body fat, visceral fat and BMI of each participant. A mercury sphygmomanometer was used to measure blood pressure of the participants after resting for at least 10 min.

Blood sampling and analyses

A volume of 7 ml of venous blood was taken from each participant after overnight fast, and the sample was divided into gel separator and ethylenediaminetetraacetic acid (EDTA) tubes. The blood in the gel separator tube was allowed to clot and centrifuged at 4000 rev/min for 5 min, and serum was pipetted into Eppendorf tubes for storage at −80 °C till ready for use. The serum was used for lipid profile measurements and lactate dehydrogenase activity using a chemistry analyser (Mindray BS-120, Shenzhen, China) and diagnostic reagents (DiaSys Diagnostic Systems, Holzheim, Germany), respectively. The lipid profile consisted of the following indices: total cholesterol (TC), high density lipoprotein cholesterol (HDL-c) and triglycerides (TG). The TC/HDL-c ratio, low density lipoprotein cholesterol (LDL-c), atherogenic index of plasma (AIP) [log (TG/HDL)], and TG/HDL-c were calculated. The blood samples in the EDTA tubes were mixed gently and used for haemoglobin level determination using a haematology analyser (Mindray BC 2300, Shenzhen, China). Viral load was analysed using HIV-1 Test automated machine (COBAS AmpliPrep/ COBAS TaqMan Roche, Unterhaching, Germany), and $CD4^+$ counts was determined using BD FACSCount™ Flow Cytometer (BD Biosciences, California, USA).

Haptoglobin phenotype analysis

Serum haptoglobin (Hp) phenotypes were determined by discontinuous polyacrylamide gel electrophoresis (PAGE) with haemoglobin-supplementation followed by 3,3,5,5-tetramethyl benzidine with o-dianisidine staining [18]. Briefly, 1.5 µl of 10% freshly prepared hemolysate was added to 8 µl serum, mixed thoroughly and incubated for 3 min at room temperature. The sample was loaded with 8 µl loading buffer and resolved on a 7.5% native PAGE at 220V for 60 min with running buffer containing 1.5 M Tris base at pH = 8.6. The gel was washed with distilled water and the protein was fixed with 10% trichloroacetic acid (TCA), followed by 3,3,5,5-tetramethyl benzidine with o-dianisidine and hydrogen peroxide staining. The stained Hp-Hb bands were typed by visualising under visible light.

Data analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS), version 20.0. Qualitative data were expressed as frequency and percentage, and quantitative data were presented as mean ± SD. The categorical parameters were analysed using Chi-square (χ^2) test to determine the significant difference between two groups. Spearman's correlation was used to determine the relationship between AIP and other variables in HIV/ AIDS patients. Odds ratio was used to determine the association between haptoglobin phenotypes and cardiovascular risk factors. P-value < 0.05 was considered statistically significant.

Results

Socio-demographic characteristic, clinical and biochemical parameters of the study population are shown in Table 1. The mean differences in the clinical parameters including body mass index (BMI), body fat and visceral fat between the patients on HAART and the HAART-naïve group were not statistically significant ($P > 0.05$). However, systolic blood pressure (SBP), total cholesterol (TC) and low-density lipoprotein (LDL-c) were significantly elevated in patients on HAART compared to the HAART-naïve group ($P < 0.05$). The high density lipoprotein (HDL-c) level was significantly decreased in the patients on HAART, and thus increasing the atherogenic index of plasma (AIP) ($P < 0.01$). $CD4^+$ counts were significantly higher in patients on HAART whereas viral load was significantly lower than in HAART-naïve counterparts ($P < 0.001$). The most common combined therapies used for HIV treatment were Lamivudine (3TC)-Zidovudine (AZT)-Efavirenz (EFV) (42.9%) and Lamivudine (3TC)-Zidovudine (AZT)-Nevirapine (NVP) (30.5%) (Figure 1).

Overall prevalence of the assessed risk factors of CVD among the study population ranged from 13.3% to 67.2% (Table 2). Hypertension, obesity and increased LDL-c were least presented whiles decreased HDL-c (67.2%) was the major risk factor. Overall prevalence of high AIP, hypercholesterolaemia and hypertriglyceridemia were 43.3%, 26.1% and 21.7%, respectively. Among the patients on HAART, prevalence of hypertension (19.1%), hypercholesterolaemia (35.2%), decreased HDL-c (73.3%), increased LDL-c (19.0%) and high AIP (53.3%) were significantly higher than the HAART-naïve counterparts ($P < 0.05$) (Table 2). Prevalence of obesity between patients on HAART and HAART-naïve was not statistically significant ($P > 0.05$).

Table 3 shows the correlation between AIP and the clinical and biochemical variables in the HIV patients. Age, SBP, TC, TG and TC/HDL positively correlated with AIP ($P < 0.05$). HDL-c negatively correlated with AIP ($P < 0.01$). The various haptoglobin phenotypes, based on their band patterns on polyacrylamide gel electrophoresis, and the distribution among the study population are shown in Figure 2 and Table 4, respectively. The

Table 1 Socio-demographic, clinical and biochemical parameters of the study population

Variable	HAART (N = 105)	HAART-Naïve (N = 75)	95% CI	P-value
Age (years)	45.62 ± 9.39	40.75 ± 11.81	1.75–7.99	0.002*
Sex *n* (%)				
Female	59 (56)	56 (75)	4.82–31.72[ϖ]	0.009*
Male	46 (44)	19 (25)		
SBP (mmHg)	125.39 ± 23.93	112.92 ± 18.78	5.93–19.01	<0.001*
DBP (mmHg)	73.53 ± 15.46	71.04 ± 11.89	−1.71–6.69	0.244
BMI (Kg/m^2)	24.98 ± 4.49	23.91 ± 5.69	−0.43–2.57	0.160
Body Fat (%)	26.54 ± 11.93	28.94 ± 11.66	−5.93–1.13	0.181
Visceral Fat	6.64 ± 3.35	5.97 ± 3.68	−0.37–1.71	0.206
TC (mmol/l)	4.93 ± 1.20	3.97 ± 1.14	0.61–1.31	<0.001*
TG (mmol/l)	1.50 ± 1.04	1.35 ± 0.78	−0.13–0.43	0.293
HDL-c (mmol/l)	0.78 ± 0.24	0.92 ± 0.25	−0.21 to −0.07	<0.001*
LDL-c (mmol/l)	3.29 ± 1.12	2.65 ± 1.03	0.32–0.96	<0.001*
TC/HDL-c	5.68 ± 1.96	5.69 ± 1.58	−0.54–0.54	1.000
AIP	0.23 ± 0.29	0.12 ± 0.25	0.03–0.19	0.009*
HBG (g/dl)	12.48 ± 1.75	11.75 ± 4.83	−0.28–1.74	0.156
LDH (UI/l)	405.22 ± 135.94	405.16 ± 160.64	−43.71–43.83	0.997
CD4$^+$ count (cells/mm^3)	608.12 ± 277.57	320.01 ± 208.57	213.13–363.09	<0.001*
Viral load *(cp/ml) x 10^4*	2.41 ± 1.25	32.15 ± 10.80	−31.83–27.64	<0.001*

N represents the size of populations, *n* represents gender in the sub-population. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, Body mass index; TC, Total cholesterol; TG, Triglycerides; HDL-c, High density lipoprotein cholesterol; LDL-c, Low density lipoprotein cholesterol; TC/HDL-c, atherogenic index; AIP, atherogenic index of plasma [log (TG/HDL)]; HBG, Haemoglobin; LDH, Lactate dehydrogenase. [ϖ]Chi square (χ^2). *P-value < 0.05 was considered statistically significant.

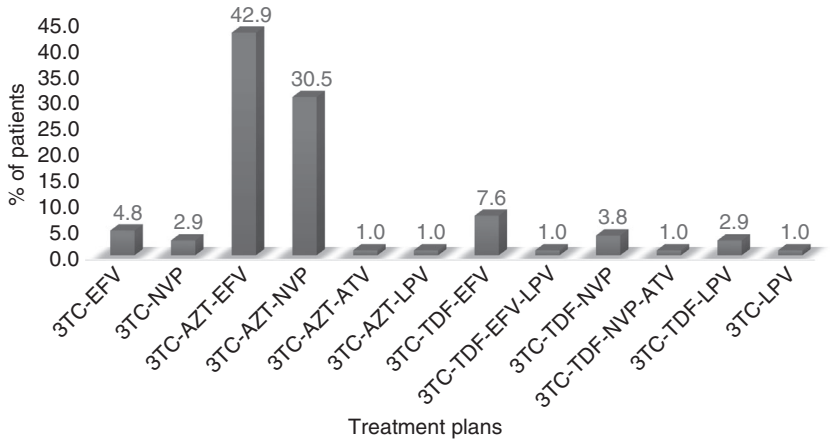

Figure 1 Percentage distribution of treatment options for HIV-infected patients. The therapies were: 3TC, Lamivudine; EFV, Efavirenz; NVP, Nevirapine; AZT, Zidovudine; ATV, Atazanavir; LPV, Lopinavir; TDF, Tenofovir.

frequencies of the Hp phenotypes among the study population showed no significant difference between the patients on HAART and the HAART-naïve group ($P > 0.05$).

The haptoglobin phenotypes distribution was determined from the band patterns of the polyacrylamide gel electrophoresis. Comparison of the distribution was determined using Chi-square (χ^2). A P-value < 0.05 was considered statistically significant.

Comparison of risk factors of CVD in HIV patients to the Hp phenotypes is shown in Table 5. Generally, patients with weak antioxidant Hp phenotypes (Hp2-2 and Hp0) showed elevated clinical and biochemical parameters compared to patients with strong antioxidant Hp phenotype (Hp1-1 and Hp2-1). Patients on HAART expressing the weak antioxidant Hp phenotypes showed elevated SBP and BMI compared to patients expressing the strong antioxidant Hp phenotypes ($P < 0.05$).

Table 2 Risk factors of cardiovascular disease among HIV-infected patients

Risk factors of CVD	Overall prevalence (N = 180)	Prevalence in patients on HAART (N = 105)	Prevalence in HAART-naïve patients (N = 75)	χ^2 (95% CI)	P-value
Hypertension, *n* (%)	26 (14.4)	20 (19.1)	6 (8.0)	4.33 (0.58–20.67)	0.037*
Obesity, *n* (%)	27 (15.0)	17 (16.2)	10 (13.3)	0.31 (−8.07 to 13.05)	0.577
Hypercholesterolaemia, *n* (%)	47 (26.1)	37 (35.2)	10 (13.3)	10.99 (9.33–33.15)	<0.001*
Hypertriglyceridemia, *n* (%)	39 (21.7)	25 (23.8)	14 (18.7)	0.64 (−7.54–16.57)	0.425
Decreased HDL-c, *n* (%)	121 (67.2)	77 (73.3)	44 (58.7)	3.87 (0.100–27.60)	0.049*
Increased LDL-c, *n* (%)	24 (13.3)	20 (19.0)	4 (5.3)	7.45 (4.18–23.09)	0.006*
High AIP, *n* (%)	78 (43.3)	56 (53.3)	22 (29.3)	10.22 (9.41–36.95)	0.001*

N represents the size of populations of the subgroup, *n* is the number of patients in the subgroup showing the CVD risk factors. Hypertension: blood pressure > 140/90 mmHg, obesity: body mass index; body weight > 30 kg/m^2, hypercholesterolaemia: cholesterol level > 5.2 mmols/l, decreased-HDL-c: high density lipoprotein level < 1.0 mmol/l, increased-LDL: low density lipoprotein level > 4.12 mmol/l, hypertriglyceridemia: serum triglyceride level > 1.8 mmol/l, AIP: atherogenic index of plasma [log (TG/HDL)], high AIP > 0.24. Comparison was between patients on HAART and the HAART-naïve group. **P*-value < 0.05 was considered statistically significant.

Table 3 Correlation between atherogenic index of plasma and clinical and biochemical variables in HIV/AIDS patients

Variable	Spearman's correlation coefficient (R)	P-value
Age	0.196	0.008*
BMI	0.144	0.054
Body fat	−0.078	0.289
Visceral fat	0.144	0.055
SBP	0.154	0.039*
DBP	0.083	0.270
Hb	0.043	0.569
CD4$^+$	0.007	0.929
LDH	0.116	0.121
TC	0.240	0.001*
TG	0.859	<0.001*
HDL	−0.660	<0.001*
LDL-c	0.104	0.167
TC/HDL	0.626	<0.001*

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TC/HDL-c, atherogenic index; HBG, haemoglobin; LDH, lactate dehydrogenase. **P*-value < 0.05 was considered statistically significant.

Association of weak antioxidant Hp phenotypes (Hp2-2 and Hp0) with risk factors of CVD in patients on HAART is shown in Table 6. The weak antioxidant phenotypes were strongly and significantly associated with hypertension (OR = 2.54; P = 0.011), obesity (OR = 5.97; P < 0.001) and hypercholesterolaemia (OR = 2.99; P < 0.001). Even though the association between increased LDL-c, atherogenic index and AIP were not statistically significant, patients expressing Hp phenotypes with weak antioxidant properties were high with the exception of hypertriglyceridemia.

Discussion

Introduction of HAART in the management of HIV/AIDS has resulted in a decline in AIDS-related mortality globally [3, 5]. Selection of combined inhibitors that have been approved for combating HIV infection largely depends on the clinical outcomes of the patients [25, 26]. Previous treatment plans depended on the CD4$^+$ count and viral loads of the patients. Currently, WHO recommens treatment for all HIV patients regardless of the CD4$^+$ count [7]. First-line drugs consist of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitors (NNRTI); second-line drugs consist of two NRTIs and ritonavir-boosted protease inhibitor (PI), and addition of medicines with less risks for the development of cross-resistance towards the first- and second-line drugs is used as third-line therapy [7]. Combination therapy must target the reduction in co-morbidities that include, but are not limited to, CVD, hyperlipidemia, renal disease and neurological disease [27]. Assessment of the risk factors of co-morbidities in HIV patients will help in the selection of appropriate therapy to reduce treatment-associated complications. The impact of Hp phenotypes on dyslipidemia and other risk factors of CVD in Ghanaians infected with HIV was investigated.

In this study, greater proportion of the study participants were on the first-line drugs (Figure 1); lamivudine (3TC) and zidovudine (AZT) combined with nevirapine (NVP) as one option while the other option was 3TC

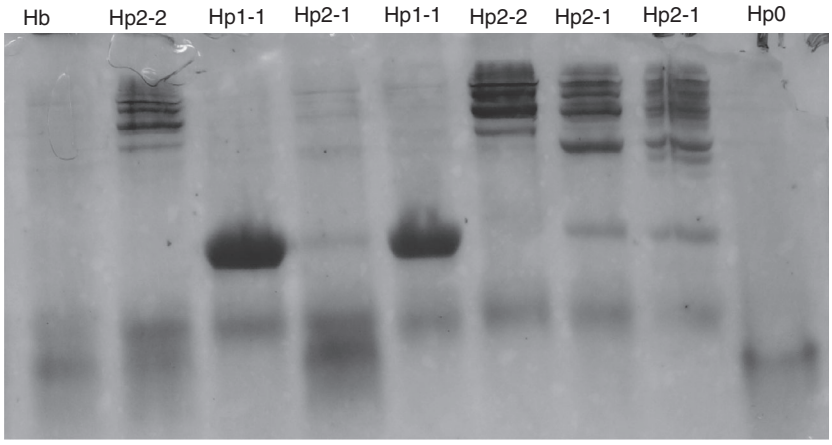

Figure 2 Polyacrylamide gel electrophoresis of haptoglobin phenotypes. The haptoglobin phenotypes were separated on a 7.5% polyacrylamide gel using iron-supplementation method followed by o-dianisidine and benzidine staining.

Table 4 Haptoglobin phenotypes (Hp) distribution in HIV-infected patients

	Hp phenotype			
Participant	Hp0	Hp1-1	Hp2-1	Hp 2-2
HAART (n = 105)	8 (7.6)	31 (29.5)	45 (42.9)	21 (20.0)
HAART-naïve (n = 75)	5 (6.7)	14 (18.7)	36 (48.0)	20 (26.7)
χ^2	0.06	2.77	0.44	1.21
95% CI	−7.96–8.84	2.00–22.84	−9.51–19.34	−5.29–19.77
P-value	0.803	0.096	0.508	0.272

and AZT in combination with efavirenz (EFV). The treatment resulted in improved mean $CD4^+$ counts and significantly decreased viral load in patients on HAART compared to the HAART-naïve group (Table 1). Despite the success in controlling HIV infection with HAART, reports have implicated the treatment with adverse effects [28]. Dyslipidemia was the common traditional cardiovascular risk factor that was found in the patients on HAART compared with the HAART-naïve group. The significant elevations of TC, LDL-c, and SBP, with high prevalence of hypertension suggest an increased CVD in patients on HAART. This study supports previous findings that associated hypertension and dyslipidemia with HAART administration [29–31]. A unit mmol/l increase in TC in HIV-infected individuals on HAART was found to increase the relative risk of myocardial infarction by a factor of 1.26, and the increase was attributed to dyslipidemia [32, 33]. However, the mechanism underlying the increased risk of CVD in patients on HAART is not clear [34, 35]. Viral infection has been reported to induce dyslipidemia via increased cytokine levels, increased hepatic lipid synthesis of very low-density lipoprotein (VLDL) and decreased lipids clearance [36, 37]. In keeping with increased risk of CVD due to HAART administration, atherogenic index of plasma (AIP) was also shown to be significantly high in the patients on treatment compared to the HAART-naïve group, and the AIP correlated positively with age, SBP, TC and TG. Increased AIP is dependent on altered serum lipids levels (elevated TG and lowered HDL-c levels), and is an independent predictor of CVD [38].

HIV-infected patients with genetic predisposing factors of CVD and on HAART will be at higher risk of developing CVD than the HAART-naïve patients [39]. This study investigated the possible effect of weak antioxidant haptoglobin phenotype on the development of CVD in HIV-infected patients on HAART. There was an increase in levels of CVD risk factors in HIV-infected patients on HAART, and patients expressing Hp phenotypes with weak antioxidant activities were at greater risk than patients showing phenotypes with strong antioxidant activities. Despite the observation that HAART is associated with CVD risk factors in HIV patients, it has been established that the antiretroviral therapy has considerably improved patients' health. Therefore, supplementing HAART with antioxidants will reduce risk factors for CVD in HIV patients. The weak antioxidant Hp phenotypes were strongly and positively associated with

Table 5 Haptoglobin phenotypes and risk factors of cardiovascular disease among HIV patients

Parameters	Patients on HAART			HAART-naïve patients		
	Hp phenotype (Hp1-1 and Hp2-1) (n = 76)	Hp phenotype (Hp2-2 and Hp0) (n = 29)	P-value	Hp phenotype strong (Hp1-1 and Hp2-1) (n = 50)	Hp phenotype (Hp2-2 and Hp0) (n = 25)	P-value
SBP (mmHg)	117.83 ± 19.72	133.14 ± 25.30	<0.001*	112.16 ± 17.18	115.72 ± 22.02	0.444
DBP (mmHg)	71.87 ± 15.24	77.68 ± 15.75	0.087	71.20 ± 9.92	71.44 ± 15.75	0.935
BMI (Kg/m^2)	24.38 ± 3.90	26.77 ± 5.35	0.013*	23.47 ± 6.65	24.02 ± 5.42	0.721
Visceral fat	6.27 ± 3.22	7.89 ± 3.53	0.065	5.66 ± 3.83	5.80 ± 3.33	0.876
TC (mmol/l)	4.80 ± 1.21	5.28 ± 1.15	0.068	4.04 ± 1.40	3.80 ± 0.94	0.441
TG (mmol/l)	1.37 ± 0.67	1.44 ± 0.67	0.633	1.31 ± 0.95	1.37 ± 0.82	0.788
HDL-c (mmol/l)	0.79 ± 0.22	0.80 ± 0.29	0.849	0.98 ± 0.29	0.96 ± 0.25	0.769
LDL-c (mmol/l)	3.15 ± 1.14	3.63 ± 1.01	0.095	2.67 ± 1.03	2.75 ± 1.13	0.756
TC/HDL-c	6.60 ± 2.62	7.65 ± 3.68	0.105	5.20 ± 1.40	5.76 ± 1.72	0.135
AIP	0.21 ± 0.27	0.24 ± 0.30	0.622	0.17 ± 0.26	0.24 ± 0.33	0.319

n represents the number of participants in the sub-populations. Hp1-1 and Hp2-1 are strong antioxidant haptoglobin phenotypes, and Hp2-2 and Hp0 are weak antioxidant haptoglobin phenotypes. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; TC/HDL-c ratio, atherogenic index; AIP, atherogenic index of plasma [log (TG/HDL-c)]. *P-value < 0.05 was considered statistically significant.

Table 6 Association of cardiovascular risk factors with weak antioxidant haptoglobin phenotypes in HIV-infected patients on HAART

Risk factors of CVD	Hp phenotype [n (%)] (Hp1-1 and Hp2-1) (N = 76)	Hp phenotype [n (%)] (Hp2-2 and Hp0) (N = 29)	OR (95% CI)	P-value
Hypertension	11 (14.5)	9 (31.0)	2.54 (1.19–3.58)	0.011*
Obesity	5 (6.6)	9 (31.0)	5.97 (2.05–9.58)	<0.001*
Hypercholesterolaemia	22 (28.9)	16 (55.1)	2.99 (1.67- 5.37)	<0.001*
Increased-LDL-c	17 (22.4)	8 (27.6)	1.38 (0.72–2.62)	0.414
Hypertriglyceridemia	18 (23.9)	6 (20.7)	0.84 (0.43–1.64)	0.735
Atherogenic index	49 (64.0)	19 (66)	1.09 (0.61–1.95)	0.882
AIP	40 (52.6)	17 (58.6)	1.28 (0.73–2.23)	0.476

N represents the sub-populations of patients on HAART. n represents the proportion of the condition of the patients. Hp1-1 and Hp2-1 are strong antioxidant haptoglobin phenotypes, and Hp2-2 and Hp0 are weak antioxidant haptoglobin phenotypes. Hypertension: blood pressure > 140/90 mmHg, obesity: body mass index ≥ 30 kg/m^2, hypercholesterolaemia; serum cholesterol > 5.2 mmo/l, increased-LDL-c: low density lipoprotein level > 4.12 mmol/l, hypertriglyceridemia; serum triglyceride level > 1.8 mmol/l, atherogenic index: TC/HDL-c > 5.0, AIP > 0.24. An individual could present with more than one risk factor of CVD. *P-value > 0.05 was considered statistically significant.

hypertension, obesity and hypercholesterolaemia. Haptoglobin (Hp) is a polymorphic acute phase glycoprotein which is important in inflammatory response and clearance of free circulatory iron (Fe^{2+}), and the protein clearance activity depends on the phenotype of the individual [16]. Defective clearance of Fe^{2+} leads to accumulation of other free radicals via the Fenton's reaction, and eventually results in oxidative stress with associated complications [40]. Indeed, a number of studies have associated Hp phenotypes with the development or progression of diseases, and the antioxidant activities of the phenotypes have been proposed as the underlying mechanism of the association [41]. In type 2 diabetes, Hp2-2 was considered to be a major susceptible gene for the development of coronary artery disease (CAD); it correlates with increased levels of inflammatory response markers [21, 42, 43]. The Hp2-2 phenotype has been implicated in increased HIV viral titres compared to the other Hp phenotypes [19].

Conclusion

This study is the first to report on the possible role of haptoglobin phenotypes in the development of

cardiovascular disease in Ghanaian HIV-infected patients on highly active antiretroviral therapy (HAART). Patients on HAART who are expressing Hp phenotype with weak antioxidant activity were at a higher risk of developing CVD. Therefore, determination of the traditional CVD risk factors including Hp phenotypes will provide a comprehensive assessment for HIV/AIDS management.

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