Plasma Ascorbic Acid, Lipids and Lipoproteins in HIV Infected Patients

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Absract: Human Immunodeficiency Virus induces a wide array of biochemical and immunological alterations resulting in the progressive impairment of cellular functions, several metabolic abnormalities and eventual development of acquired immune deficiency syndrome (AIDS). There is paucity of information in the plasma ascorbic acid concentration in HIV patients on antiretroviral therapy in this community. This study was designed to assess the interplay of ascorbic acid, lipids and lipoproteins in HIV positive patients on antiretroviral therapy. One hundred subjects consisting of fifty HIV positive patients with mean age of 34.02 ± 0.95 years and fifty controls with mean age of 35.06 ± 0.98 years were selected for this study. Plasma ascorbic acid, lipids and lipoproteins were estimated using spectrophotometric methods. The anthropometric indices were measured using standard methods. The result shows significant decreased in plasma HDLC ($p<0.01$) and body weight ($p<0.05$) when compared with the controls. There were significant increases in the plasma total cholesterol and LDLC ($p<0.01$) when compared with the control values. The increase in plasma triglyceride and decrease in ascorbic acid were not statistically significant when compared with the control values. There were significant correlations between TC and HDLC ($r=0.427$, $p<0.01$) and LDLC ($r=0.975$, $p<01$).TC/HDLC was negatively correlated with TC/LDLC ($r=-0.782$, $p<0.01$) and HDLC/LDLC ($r=-0.742$, $p<0.01$). The main findings are significant increases in plasma TC, LDLC ($p<0.01$), decreases in body weight ($p<0.01$) and plasma HDLC ($p<0.05$) in our HIV positive patients on antiretroviral therapy. The mean increase in plasma ascorbic acid did not reach a significant level. (Ebesunu Maria, Finebone Patience, Adetunji Kehinde, Umahoin Kingsley. Plasma Ascorbic acid, Lipids and Lipoprotein in HIV infected patients).

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1. Introduction

Human Immunodeficiency Virus is a retrovirus that attacks a specific type of white blood cells known as T-Lymphocytes, which is important in the formation of antibodies. The CD+T-Lymphocytes are the primary targets of HIV infection because of the affinity to the CD+4 surface markers. Infection with HIV leads to a progressive impairment of cellular functions characterized by a gradual decline in peripheral blood CD+4 T-Lymphocyte levels. This results in an increasing susceptibility to a wide variety of opportunistic viral, bacterial, protozoan and fungal infections (Ball and Fowke, 2003)

Lipid a diverse and ubiquitous group of compounds that have many key biological functions, such as acting as structural components of cell membranes, serving as energy storage and participating in signaling pathways (Fashy and Brown, 2005). An increased level of triglyceride could also block blood vessels and cause complications such as abnormal pain, pancreatitis and atherosclerosis (Fashy and Brown, 2005). Study has associated HIV infection with increased plasma triglyceride levels by decreasing the clearance of circulating lipoproteins; a process considered to be due either to reduced lipoprotein lipase or by stimulating hepatic synthesis or re-esterification of fatty acids derived from lipolysis (Grunfeld and Kotler 1991).Ascorbic acid, an antioxidant nutrient, serves as a potent free radical scavenger; it prevents the peroxidation of lipids and protects DNA from the damage caused by the free radicals (Graby and Singh 1991). Ascorbic acid assists the immune system to rid the body of foreign invaders. It accomplishes these by stimulating the production of white blood cells, primarily neutrophils; which attack foreign antigens such as bacteria and viruses. It boosts the body's production of antibodies (Moses and Jack 1998) and plays significant role in the synthesis of neurotransmitter, nor-epinephrine and carnithine which is involved in the metabolism of cholesterol to bile acids (Allard and Chau 1998).

Report has shown that excessive free radicals are produced in HIV infection; which progressively overwhelms the body antioxidant nutrients resulting in the development of oxidative stress and eventual

tissue damage (Papadopulos-Eleopulos et al.1995 This study was designed to determine the plasma ascorbic acid, lipids and lipoproteins in HIV infected patients on antiretroviral therapy.

2. Materials and Methods

***Subjects*:** A total of fifty patients (20 males and 30 females) between 25-45 years who have been diagnosed as HIV positive on pre highly active antiretroviral therapy were randomly selected for this study. Fifty apparently healthy HIV negative volunteers within the same age range (33 males and 17 females) were included as controls. The diagnosis of HIV was based on laboratory and clinical assessments. Ethical approval was obtained from the University of Ibadan/University College Hospital Ethical Review committee. Inform consent was obtained from each participant to take part in the study.

Anthropometric Measurement: The weight and height of all subjects were measured using standard digital weighing scale (Seca GMBH co. Germany) with a meter rule for height measurement. The body mass index was calculated using the formula proposed by Quotelet (Rowland 1989).

***Sample Collection*:** Venous fasting blood sample was collected from each patient and control into Ethylenediaminetetraacetic acid bottle. The samples were centrifuged within one hour of collection and the plasma separated into clean dry specimen tubes. The plasma was immediately analyzed for the ascorbic acid content while the remaining was stored at -20º C until analyzed for total cholesterol, high density lipoprotein cholesterol and triglyceride.

***Method of Analysis*:** Analysis of vitamin C (Ascorbic acid) was based on the method postulated by Kyaw (Kyaw 1978). This is based on the principle of the formation of a light-blue supernatant when ascorbic acid reacts with sodium tungstate in an alkaline solution; the intensity of the colour produced was proportional to the concentration of ascorbic acid in the plasma. Total cholesterol was estimated by enzymatic method of Allain et al, (Allain et al. 1973). Triglyceride was estimated by enzymatic method of David and Buccolo (Buccolo and David 1973). High density lipoprotein cholesterol was estimated by precipitating low density lipoprotein cholesterol; very low density lipoprotein cholesterol and chylomicron fractions using phosphotungistic acid in the presence of magnesium ions. The HDL cholesterol which remains in the supernatant was estimated using the total cholesterol method. Low density lipoprotein cholesterol was calculated using Friedwald et al formula (Friedwald et al, 1972).

Accuracy and precision of biochemical tests were monitored by including commercial quality control samples within each batch of test assay.

Statistical Analysis

All data were subjected to statistical analysis using statistical package of social sciences (SPSS). Student's-test was used to compare two means and Cox correlation coefficient was used to assess association between biochemical and biophysical parameters. Differences were regarded as significant at $p<0.05$.

3. Results

Table 1 shows the biophysical and biochemical parameters of all the subjects. The age, height and body mass index were not significantly different from the corresponding control values. The body weight was significantly reduced in the human immunodeficiency virus positive patients compared with the control value ($p<0.05$). There were significant increases in the plasma total cholesterol and low density lipoprotein cholesterol ($p<0.01$) when compared with corresponding control values. Although the plasma triglycerides were slightly higher in the human immunodeficiency virus positive patients, this increase was however not statistically significant when compared with the corresponding control values. There was a significant decrease in the plasma high density lipoprotein cholesterol ($p<0.01$) in human immunodeficiency virus positive patients when compared with corresponding control values. The mean plasma ascorbic acid was slightly lower in the human immunodeficiency virus positive patients. This increase was however not statistically significant.

Table 2 shows Cox's correlation coefficient of all parameters in human immunodeficiency virus positive patients. Age was a significantly correlated with body weight ($r=0.332$, $p<0.05$) and BMI($r=0.296$, $p<0.05$) while body weight was significantly correlated with height ($r=0.432$, $p<0.01$). TC was significantly correlated with HDLC ($r=0.427$, $p<0.01$) and LDLC ($r=0.975$, $p<0.01$). TC/HDLC was negatively correlated with TC/LDLC ($r=-0782$, $p<0.01$) and HDLC/LDLC ($r=-0.742$, $p<0.01$).No significant correlations were obtained in the other parameters.

Table 1: Biophysical and biochemical parameters in all subjects (Mean ± SEM)

Variables	Patients (n=50)	Controls (n=50)	t-value	p-value
Age (Yrs)	34.02 ± 0.95	35.06 ± 0.98	-0.76	NS
Weight (Kg)	57.32 ± 1.70	70.38 ± 5.90	-2.12	<0.05
Height (m)	1.61 ± 0.01	1.63 ± 0.01	-1.72	NS
BMI (Kg/m²)	22.15 ±0.58	26.61 ± 2.44	-1.78	NS
Ascorbic acid (mg/dl)	0.40 ± 0.03	0.49 ± 0.02	-1.22	NS
TC(mg/dl)	187.60 ±13.80	134.74 ± 4.60	3.64	P<0.01
TG(mg/dl)	140.04 ±10.70	129.84 ± 8.40	0.75	ns
HDLC(mg/dl)	38.68 ± 2.60	58.18 ± 2.90	-5.04	P<0.01
LDLC(mg/dl)	120.97 ±12.60	50.59 ± 4.40	5.26	P<0.01
TC/HDLC	0.57 ± 0.03	0.36 ± 0.02	5.67	P<0.01
HDLC/LDLC	3.75 ± 0.50	0.36 ± 0.02	5.14	P<0.01

HDLC = High Density Lipoprotein Cholesterol
Yrs = Years
BMI = Body Mass Index
NS = Not Significant
SEM = Standard Error of Mean
AA = Ascorbic Acid
TC = Total Cholesterol
NS = Not Significant
TG = Triglyceride

Table 2: Cox's correlation coefficient of physical and biochemical parameters in HIV positive patients.

	Age (Yrs)	Weight (Kg)	Height (m²)	TC (mg/dl)	HDLC (mg/dl)	LDLC (mg/dl)	BMI (kg/m²)	TC/HDLC	TC/LDLC	HDLC/LDLC
Age (Yrs)	.332*						.296*			
Weight (Kg)	.332*		.432**				.863**			
Height (m²)	.432*									
TC (mg/dl)					.427**	.975**				
HDLC (mg/dl)				.427*						
BMI (kg/m²)	.296*	.863**								
LDLC (mg/dl)				.975**						
TC/HDLC									-.782	-.742
TC/LDLC										
HDLC/LDLC									-.782	.644**

TC = Total Cholesterol
HDLC = High Density Lipoprotein Cholesterol
LDLC = Low Density Lipoprotein Cholesterol
BMI=Body mass index
Yrs = Years

4. Discussion

The body weight was significantly reduced in the human immunodeficiency virus positive patients. This change may be attributed to viremia; which could have contributed to decrease in immune response and this invariably may have accounted for the reduced body weight. This finding is similar with a previous report (Behrens et al. 2000) which showed apparent reduction in body weight in HIV patients due to loss of subcutaneous fat.

Although, there was a decrease in the mean plasma ascorbic acid value, this decrease was however not significant. This slight decrease may suggest a gradual depletion of cellular antioxidant in these patients that in part regulates the free radicals generated during oxidative stress which expectedly

could be overwhelming in these patients. It could be speculated that the insignificant increase in plasma ascorbic acid, may be due to increase awareness among our patients on the essentials of dietary supplementation of ascorbic acid.

The plasma total cholesterol and low density lipoprotein cholesterol were significantly different from the controls, the positive correlation between total cholesterol and low density lipoprotein cholesterol indicates that increase in low density lipoprotein can lead to corresponding increase in total cholesterol. This may suggest predisposition of HIV patients to cardiovascular disease and peripheral disease risk; since increase in low density lipoprotein cholesterol is a possible index of atherosclerosis risk (Fashy and Brown 2005). The TC/LDLC was significantly higher in the patients than the controls. An earlier study has shown that increase level of this is a strong marker of atherogenic index (Behrens et al, 2000).

The decrease in the mean HDLC of HIV patients as obtained in this study is an indication of potential risk of CVD in these patients. An earlier report by Grunfeld et al, (Grunfeld and Kotler 1991) showed reduced level of plasma HDLC in HIV infected patients. This suggests that these patients are more likely prone to developing premature cardiovascular disease in which reduced plasma HDLC in a part. Plasma triglyceride, a known independent risk factor for cardiovascular disease was not significantly increased in HIV positive patients when compared with the controls.

5. Conclusion

` The study has demonstrated a decrease in plasma ascorbic acid, HDLC, body weight and body mass index in all the HIV positive patients.

Plasma ascorbic acid, lipids and lipoproteins were altered in our human immunodeficiency virus infection patients on antiretroviral therapy. However, further study is warranted.

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There are no conflicts of interest among the authors.

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