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Original Article

Variations in plasma lipids and lipoproteins among cardiovascular disease patients in South-western Nigerians

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ABSTRACT: This study was designed to assess the changes in plasma lipids and lipoproteins, in particular high-density lipoprotein (HDLC) in patients suffering from different types of cardiovascular disease (CVD) in South-western Nigeria. Patients were drawn from different socioeconomic classes in order to determine the effect of this factor on CVD in Nigeria. One hundred and seventy (74 males, 96 females) CVD patients (hypertensive heart disease (n=48), hypertension (n=59), ischaemic heart disease (n=49), myocardial infarction (n=4)) with a mean age of 45.3±13.2 years were selected. Fifty-eight individuals (31 males and 27 female) with mean age of 44.8±11.7 years were included as controls. Result showed significant increases in mean plasma total cholesterol (TC) ($P < 0.05$), triglyceride and low density lipoproteins (LDLC) ($P < 0.01$), LDLC: HDLC ($p < 0.001$), as well as in systolic and diastolic blood pressures ($p < 0.001$), while plasma HDLC ($p < 0.01$) and HDLC:TC ($p < 0.001$) showed significant decreases when compared with the corresponding mean control values. There were graded decreases in plasma HDLC in the different socioeconomic classes. The lowest mean plasma HDLC was found in IHD patients. This study has uncovered variations in plasma lipids and lipoproteins among patients of CVD within the different socioeconomic classes in South-western Nigeria.

KEYWORDS: HDL cholesterol, cardiovascular disease, plasma lipids and lipoproteins, South-western Nigeria.

INTRODUCTION

Premature cardiovascular disease is emerging as a major cause of death in developing countries (Beaglehole, 1992). Among the principal markers of increased risk of CVD are raised plasma total cholesterol and low density lipoproteins as well as decreased high density lipoprotein. Whether these are related to social class differences and subtypes of CVD in general is uncertain, but few studies have shown an association between socioeconomic classes and plasma lipid and lipoprotein changes (Hesis *et al.*, 1980, Wamala *et al.*, 1997; Darmon and Drewnowski, 2008). These earlier studies have however neither considered the socioeconomic status and their relevance in different subtypes of CVD. Some studies have indicated that socioeconomic status (SES) is inversely associated with increased CVD risk factors and diseases, (Leupker *et al.*, 1993; Wamala *et al.*, 1997) whereas others have reported a positive association (Woodward *et al.*, 1992).

In Nigeria CVD has become one of the major causes of death especially in urban areas (Akinkugbe, 1976). An earlier study by Taylor and Agbedana (1983) indicated that circulating low level of plasma high density lipoprotein cholesterol (HDLC) was positively associated with SES in free living individuals. Similarly, in Swedish free-living women aged 30-65 years, (Wamala *et al.*, 1997) reported that plasma HDLC levels were associated with low SES. Detailed studies on possible

relationship between SES and circulating lipoproteins in patients with subtypes of CVD are very scanty in the Nigerian African populations. It is thought that the changing patterns of CVD risk factors may be related to increasing adoption of Westernized lifestyle by different Nigerian African populations.

The aim of this study was to determine plasma lipids and lipoproteins profiles, in particular low HDLC and increased LDLC: HDLC ratio in patients suffering from different forms of CVD in a Nigeria community. In addition, we assessed the effects of socioeconomic status on these biochemical parameters that are relevant to cardiovascular diseases.

MATERIALS AND METHODS

Subjects

One hundred and seventy (74 males, 96 females) patients suffering from different types of CVD made up of hypertensive heart diseases (HHD) (n=48). Hypertension (HT) (n=59), ischaemic heart diseases (IHD) (n=49), myocardial infarction (MI) (n=14) as diagnosed by the attending Consultant Cardiologist were randomly selected at the Cardiac Unit of the Department of Medicine University College Hospital Ibadan, Nigeria. The patients were aged between 20-51 years with a mean age of 45.3± 13.2 years. The random selection was drawn out of a total of 480 patients who were either attending clinic or on admission in the cardiac ward unit of the University College Hospital Ibadan. Data from the completed questionnaire indicating the educational level, occupation, income (earning) and living conditions such as ownership of material goods were used as a measure of socio-economic status to classify the subjects into low (petty traders, famers, artisans, cleaners and junior staff in public offices (with primary/secondary education), middle (workers in the senior level cadre with university or polytechnic/higher education) and high income (executive business class, captain of industries. and directors of institutions) groups as adopted by Ebesunun *et al*., (2008) in an earlier study in this community. A written/oral informed consent was obtained from each patient to participate in the study. Fifty-eight (31 males and 27 female) healthy volunteers (aged between 19-48 years with mean 44.8±11.7 years) from the same community were selected as controls after medical examination by the attendant Consultant Cardiologist. The random selection was drawn out of a total of 120 individuals. Those with history of diabetes, liver disease, renal dysfunction and any other disease conditions that could affect the outcome of the results were excluded from the study. The control subjects were classified as low, middle and high-income groups using the same criteria as for the patients. Ethical approval was obtained from the Medical Ethics Committee of the University of Ibadan and the University College Hospital, Ibadan, Nigeria. The size of the sample was estimated based on the 10% prevalence rate of CVD in Nigeria (Akinkugbe, 1976)

Methods

Blood samples were drawn in the morning after an overnight fast (10–14 hours) into EDTA bottles (1 mg/ml blood) and immediately placed on ice bag until centrifuged. Plasma samples were collected after centrifugation and stored at –70 ^{0}C until analysed. Total cholesterol was analysed based on the method of Allian *et al*., (1974), High-density lipoprotein was determined after the isolation of LDLC and VLDLC from serum by precipitation. The cholesterol content of the supernatant cholesterol was measured enzymatically based on the method of Allian *et al*., (1974). The plasma triglyceride was estimated using an enzymatic colorimetric procedure based on the method of Buccolo and David (1973). The low density lipoprotein cholesterol was calculated using the Friedwald *et al*., (1972), formula (LDLC (mg/dl)=TC-(HDLC+TG/5).

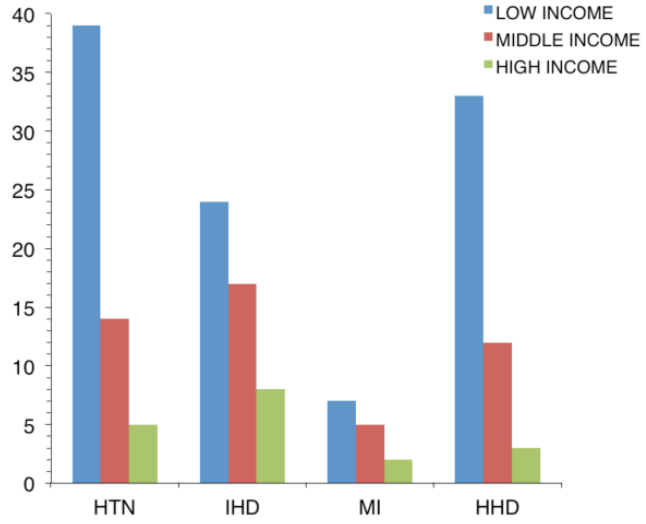

Figure 1: Distribution of all subjects within the socio-economic classes. HTN= Hypertension; IHD= Ischaemic heart disease; MI=Myocardial infarction; HHD= Hypertensive heart disease.

The accuracy and precision of all biochemical tests were monitored using commercial quality control samples within each batch of test assay.

Blood pressure was measured on the left arm while the subjects were seated or ambulant (average of two readings were taken), weight and height were measured after the interview. Height was measured without shoes and corrected to the nearest millimetres. Weight was measured with light clothing on and without shoes. The percentage body fat was calculated from the measured skinfold thickness values.

Relative body fat

The percentage body fat was calculated using the MOHR equation (Cole and Udekwe, 1989)

%BF = - 20 + 17.305 x log *x*1 + 12.012l log*x*2 +6.293 x log*x*3

*x*1 = Skinfold triceps (mm)

*x*2 = Skinfold Subscapular (mm)

*x*3 = Skinfold Iliac crest (mm) (Cole and Udekwe,1989)

Statistical analysis

All results were expressed as means plus standard error of mean (X ± SEM). Student t-test and multivariate analysis of variance (ANOVA) were used for statistical comparisons using SPSS package. The difference was regarded as significant at $p < 0.05$.

RESULTS

Figure 1 shows distribution of all subjects within the different socio-economic classes. There was a preponderance of patients suffering from the different types of CVD in the low-income group. The relative distributions of low, middle and high-income subject was 37:44:4, 23:17:8, 7:5:2 and 32:12:3 for HT, IHD, MI and HHD respectively. The relative proportion of the corresponding control group was 15:13:1.

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

Variables	Patients (n=173)	Control (n=58)	t-value	p-value
TC (mg/dl)	185.70±42.30	169.09±46.77	1.962	0.001
HDLC (mg/dl)	36.43±13.60	46.81±11.7	4.100	0.01
TG (mg/dl)	98.39±3.57	85.21±28.8	2.204	0.01
LDLC (mg/dl)	130.03±4.03	105.63±4.04	2.967	< 0.01
HDLC: TC	0.21±0.01	0.29±0.08	5.551	< 0.001
LDLC:HDLC	4.52±0.42	2.45±0.11	4.60	< 0.001
SBP (mmHg)	148±3.00	115±2.38	8.799	< 0.001
DBP (mmHg)	89±1.23	75.46±1.70	5.750	< 0.001
BMI (kg/m^2)	27±0.40	26.00 ±0.57	0.417	NS
%BF	20.49±0.30	19.43±0.02	1.885	< 0.05

TC=Total cholesterol; HDLC=High density lipoprotein cholesterol; LDLC=Low density lipoprotein cholesterol; TG=Triglyceride; NS=Not significant; n=Number of subject; SE=Standard error.

Table 1 show the biophysical and biochemical parameters in all subjects. There were significant changes in the mean plasma TC ($p < 0.05$), HDLC, HDLC:TC and LDLC: HDLC ($p < 0.001$) as well as plasma TG ($p < 0.01$) in all the patients when compared with the corresponding mean control values. There were also significant increases in the mean systolic and diastolic blood pressure ($p < 0.001$). The %BF was significantly increased ($p < 0.05$) when compared with the corresponding mean control value.

Table 2: Biochemical parameters in male and female patients and controls (Mean ± SEM)

	Patients		Control	
Variables	Male (n=74)	Female (n=96)	Males (n=31)	Female (n=27)
SBP (mmHg)	146±3.07**	149±2.70**	114±3.58*	116±3.15**
DBP (mmHg)	91±1.83**	87±1.00**	75±1.63**	75±1.321**
BMI (Kg/m^2)	26.66±0.61	27.52±0.56	26.66±0.61	27.52±0.65
%BF	20.23±0.51	20.83±0.38	18.84±0.38	20.10±0.44
TC (mg/dl)	171±5.21	196±6.33	170±8.06	167±8.40
HDLC (mg/dl)	33±1.76**	38±1.99**	46.81±1.92**	48.81±2.62**
TG (mg/dl)	100±5.71	97±3.66*	89±5.69	80±4.78*
LDLC (mg/dl)	110±0.51	140±6.04	107±8.06	103±8.85
HDLC:TC	0.21±0.01**	0.22±0.01**	0,28±0.01**	0.30±0.02**
LDLC:HDLC	4.53±0.41**	4.50±0.31**	2.42±0.41**	2.49±0.35**

Level of significance: (*P < 0.01, **P < 0.001); TC=Total cholesterol CVD=Cardiovascular disease; HDLC=High density lipoprotein cholesterol; LDLC=Low density lipoprotein cholesterol; TG=Triglyceride; NS=Not significant; n=Number of subject; SE=Standard error.

Table 2 shows the biophysical and biochemical parameters in male and female patients and controls. The mean SBP of the patients was higher than the corresponding sex-matched mean control value ($p < 0.001$). The BMI and: %BF showed no significant changes. The female patients exhibited an elevated mean plasma TC and LDLC ($p < 0.01$) concentrations when compared with the male patients, the male patients had a significantly higher mean plasma TG ($p < 0.01$) compared with the female controls. The male patients had lower mean plasma HDLC ($p < 0.05$) when compared with the controls. The mean plasma HDLC in the male patients was significantly lower than the level in the female controls. The mean HDLC:TC ratio was reduced in both male and female patients ($p < 0.001$) when compared with the corresponding control values in male and female control subjects respectively.

Classification of patients into socioeconomic classes showed graded decreased in plasma HDLC. The low-income class had the lowest plasma HDLC. When these changes were subjected to within group analysis, no significant changes were obtained (Table 3).

Table 4 shows the biochemical parameters in patients classified according to the disease types. Within group analysis of all parameters in the different subtypes of CVD showed significant variations in mean plasma HDLC and LDLC ($p < 0.001$) and HDLC:TC as well as LDLC: HDLC ratios ($p < 0.001$). The SBP and DBP ($p < 0.001$) were

significantly increased in all the disease types when compared with respective control values. Also the mean DBP reading was greater than the cut off pressure reading of ≥ 90 mmHg only in HT and HHD patients, while the mean SBP reading was greater than the cut off pressure reading of ≥ 140 mmHg in HT, IHD and HHD patients. The mean TC, %BF and BMI showed no significant changes in the different groups.

Table 3: Socio-economic status, biophysical and biochemical parameters in CVD patients (Mean ± SEM)

Variables	Low income (n=105)	Middle income (n=47)	High income (n=18)	F-value	P-value
SBP (mmHg)	149±2.81	145±3.20	144±0.87	0.560	NS
DBP (mmHg)	90±1.69	86±1.42	88±18.24	1.05	NS
BMI (kg/m^2)	26,9±0.95	28.3±0.83	25.7±0.86	1.73	NS
%BF	19.9±0.42	21.50.43	21.1±2.54	2.211	NS
TC (mg/dl)	183±6.1	186±8.60	192±5.58	0.182	NS
HDLC (mg/dl)	35±1.82	36.±0.94	40±19.8	0.77	NS
LDLC (mg/dl)	131±5.83	125±7.94	135±8.67	0.262	NS
TG (mg/dl)	96±3.56	100±6.27	85±4.58	0.903	NS
HGLC/TC	0.21±0.00	0.21±0.00	0.22±0.02	0.156	NS
LDLC/HDLC	4.49±0.31	4.28±0.05	5.30±1.28	0.681	NS

TC=Total cholesterol CVD=Cardiovascular disease; HDLC=High density lipoprotein cholesterol; LDLC=Low density lipoprotein cholesterol; TG=Triglyceride; NS=Not significant; n=Number of subject; SE=Standard error.

Table 5 shows the distribution of lipids and lipoproteins in all subjects. Patients with HT showed the highest increase in mean TC > 200 mg/dl, LDLC > 135 mg/dl and decrease in HDLC < 35 mg/dl in the different subtypes of CVD.

DISCUSSION

This study examined changes in lipids and lipoprotein within the different socio-economic classes in patients suffering from different types of CVD in Nigeria. The observations indicated that the subtypes of CVD exhibited variations in the mean plasma lipoprotein concentrations in the different socioeconomic groups. Of these variables, low plasma HDLC levels were more closely associated with socioeconomic status. Several large scale population studies have found an association between lipid variables and socioeconomic class in both men and women (Scottish Heart Health Study (Woodward *et al*., 1992) Minnesota Heart Survey, (Leupker *et al*., 1993). Tromso Heart Study (Jacobsen and Telle 1988), Framingham Offspring Study (Garrison *et al*., 1993) and Australian National Heart Foundation risk factor prevalence study (Simon *et al*., 1986). The tendency for low plasma HDLC was more evident in the low income group, while the highest level was obtained in the high income group. The association between low plasma HDLC and socioeconomic status found in the present study is in part in accordance with the findings from earlier studies elsewhere (Lipid Research Clinics program relevance study (Hesis *et al*., 1980), Framingham offspring study (Garrison *et al*., 1993) and Australian Study (Simon *et al*., 1986) where low plasma HDLC was obtained in unclassified normal subjects. High density lipoprotein cholesterol concentration in plasma is commonly seen as an indicator of the efficiency of the reverse cholesterol transport, and because of the inverse relationships between HDLC levels and coronary heart disease rate in different populations, plasma concentration is of immense value in atheroprotective mechanism. Stratification of patients into socio-economic groups showed that about 60% were in the low income class, while only 12% belonged to the high income class.

Table 4: Biochemical and biophysical parameters in the different types of CVD and controls (Mean ± SEM)

Variables	HT (n=59)	IHD (n=49)	MI (n=14)	HHD (n=48)	Control (n=58)	F value	P value
TC (mg dl)	166±5.56	181±5.80	171±14.5	168±6.06	169±6.14	1.795	NS
HDLC (mg/dl)	38±2.97	32±1.98	34±4.44	39±2.43	46±1.54	6.360	< 0.001
TG (mg/dl)	110±5.10	93±5.98	103±18.4	89±5.49	85±3.78	3.666	< 0.01
LDLC (mg/dl)	121±6.99	134±5.98	136±12.2	138±9.61	105±5.91	3.060	< 0.001
HDLC:TC	0.22±1.7	0.17±1.20	0.19±2.6	0.20±1.27	0.29±1.1	9.403	< 0.001
LDLC:HDLC	4.46±0.4	5.2±0.55	4.7±0.94	3.9±028	2.45±0.20	6.726	< 0.001
SBP (mmHg)	155±3.09	140±3.83	133±5.89	154±3.33	114±2.38	28.88	< 0.001
DBP (mmHg)	91±1.97	82±2.48	84±4.27	95±2.06	75±1.71	15.29	< 0.001
%BF	20.9±0.3	19.8±0.61	20.2±0.6	21.2±0.79	19.4±0.30	2.246	NS
BMI (Kg/m^2)	27.7±0.7	25.9±0.71	28.3±1.5	28.9±0.72	27.2±0.72	2.280	NS

NS=Not significant; TC=Total cholesterol; HDLC= High density lipoprotein cholesterol; LDLC=Low density lipoprotein cholesterol; TG=Triglyceride; n=number of subjects.

Table 5: Percentage distribution of lipids and lipoproteins in all subjects

Variables	MI (n=14)	HT (n=59)	HHD (n=49)	iHD (n=48)	Control (n=58)
TC<200mg/dl	50% (7)	57.6% (34)	53.1% (26)	62.5% (30)	82.7%(48)
TC>200mg/dl	50% (7)	42.3% (25)	46.9% (23)	37.5% (18)	17.3%(10)
HDLC<35mg/dl	57.1% (8)	54.2% (32)	57.1% (28)	33.1% (30)	14%(8)
HDLC>35mg/dl	42.9% (6)	45.8% (27)	42.9% (21)	27.9% (18)	86%(50)
LDLC<13mg/dl	64.3% (9)	66.1% (39)	60.4% (29)	70.8% (34)	75.9%(44)
LDLC>135mg/d	35.7% (5)	33.9% (20)	39.6% (20)	24.1% (14)	24.1%(14)

TC=Total cholesterol; HDLC=High density lipoprotein cholesterol; LDLC=Low density lipoprotein cholesterol; TG=Triglyceride; HT=hypertension; HHD= hypertensive heart diseases; MI=myocardial infraction; IHD= ischemic heart disease; n=number of subjects, actual number of patients are shown in parentheses.

The large number of patients belonging to the low income class may be one important factor responsible for the rather small increase in mean plasma TC compared to the controls. A number of studies suggest that poor living conditions in childhood and adolescence contribute to increase risk of atherosclerosis (Hirdes and Forbes 1992, Leino *et al*., 2000). There are however limited studies of lipid profile in relations to subtypes of CVD and socioeconomic class. The highest increase in mean plasma LDLC concentration was found in HHD patients. The relative increases in the plasma LDLC was more pronounced than that in the plasma TC in all the patients, with the highest value obtained in IHD patients. The extent of changes in the mean lipid profile observed in this study may be a reflection of the heterogeneous nature of the patients studied.

The mean plasma LDLC which is widely regarded as one of the most reliable risk factor for predicting the development of premature CVD in humans was slightly above acceptable reference value (> 135 mg/dl), with MI and HHD showing the highest mean values. Factors that may be important determinants of circulating cholesterol concentration include socioeconomic class (Taylor and Agbedana, 1983), body composition (Segal *et al*., 1987) and gender (Taylor and Agbedana, 1983).

This study also showed inter-group variations with graded increases in the mean plasma TC and decreases in HDLC within the three socioeconomic classes with the lowest value observed in the low income group while the highest value was seen in the high income group. Certain dietary factors known to influence the plasma level of TC may have accounted for this difference. The status may be related to higher total calorie, total fat and cholesterol intake as well as lack of physical activity. There were generalized decreases in the mean plasma HDLC within the different CVD groups with IHD patients showing the lowest value. These changes could be attributed to the severity of the disease. It is reported that more affluent Nigerians have higher plasma HDLC than the ones in the low income group (Taylor and Agbedana, 1983).

Similarly, the female group was found to have higher mean plasma HDLC and HDLC:TC ratio levels than their male counterparts. This could be as a result of the age group studied, thus supporting in part an already established notion that women of childbearing age are less prone to coronary heart disease since estrogen is known to enhance favourable level of plasma HDLC.

The relative proportion of the different lipoproteins as measured by LDLC:HDLC ratio has been reported to be more useful predictor of coronary artery disease in different populations (Segal *et al*., 1987; Taylor and Bamgboye, 1979). Furthermore, Siegrist *et al.* (1988) and Otho-Gomer *et al.* (1994) showed that adverse long lasting chronic job stress may increase the ratio of LDLC:HDLC. Stress was however not considered in this study. Although epidemiological studies demonstrated a significant relationship between risks of developing coronary heart disease and reduced plasma HDLC, the exact mechanisms by which plasma HDLC exerts this atheroprotective effect is still unresolved, but the reverse-cholesterol transport mechanisms have been commonly proposed. It could also be speculated that the same dietary factors that influence the level of plasma total cholesterol may also have a similar effect on the plasma HDLC level. An earlier report by Goldbourt *et al.* (1997) has shown that plasma HDLC < 35 mg/dl increased mortality for coronary heart disease in men, independent of the TC level.

The percentage of decreases in plasma HDLC in the subtypes of CVD from the control group showed IHD (30%),

MI (21%), HHD (18.5%) and HT (18%) respectively, is suggesting that decreased HDLC is common with all the subtypes of CVD in this study. The desirability of raising HDLC is based on multiple lines of evidence; perhaps the most direct argument is that there is increase of about 2.5% in CVD risk for each mg/dl decrease in HDLC level (Goldbourt *et al.*, 1997). In the present study 34% of the cases studied were patients with hypertension, while only 10% were MI patients. Physical activity and exercise are known to enhance plasma HDLC. Indications from the questionnaire revealed that many of these patients did not engage in any regulated physical exercise as a routine.

The HDLC:TC ratio was reported as a useful and simple index of IHD risk in men in the Quebec Cardiovascular Study (Lemieux *et al.*, 2001). It is proposed that the ability of this ratio to predict risk could be explained by the fact it is a relevant cumulative marker of the cluster of metabolic abnormalities found in individuals with high TG-low HDLC dyslipidemia. The mean plasma TG, a known independent risk factor for premature CVD, was significantly increased in the patients compared with value in the control group. It is important to note that slight intergroup variations occurred in the mean TG concentration within the disease subtypes. The highest increase in mean plasma TG was found in HT group, thus indicating a significant alteration of atherogenic lipid in this group, probably suggesting that the significant hypertriglyceridemia in all classes could be related to the constant finding of hypertension in all groups. It is noteworthy that in CVD of varying aetiology hypertriglyceridemia is a constant feature suggesting that dyslipidaemia could arise as a complication or part of the disease process (Garber and Avins, 1994)

Conclusions

HDL functions at an extravascular level, it is expected that decreases in levels of circulating HDLC as obtained in this study irrespective of mechanism, will not be able to confer protection against accelerated plaque formation in the patients. The present results indicate that decreased plasma mean HDLC is associated with the different types of CVD and varied within the socioeconomic classes.

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