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Research Article
Measurement of Cholesterol Sub-Fractions, High Density Lipoprotein 2 and High Density Lipoprotein 3 in Type 2 Diabetes Mellitus Patients in Burkina Faso (West Africa)

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Abstract

Background: According to literature, the High Density Lipoprotein 2 (HDL2) cholesterol is a better predictor of cardiovascular disease than total HDL. Thus, this study was undertaken to evaluate the interest of HDL2 in the management of Type 2 Diabetes Mellitus (T2DM) patients recruited at University Hospital Yalgado Ouedraogo of Ouagadougou (Burkina Faso, West Africa). **Methodology:** The distribution of type 2 diabetes group according to cardiovascular complications reported 37.9% with microvascular complications and 36.4% with macrovascular complications. **Results:** The total cholesterol, triglyceride, HDL cholesterol and HDL3 cholesterol were significantly higher in type 2 diabetes population compared to control group ($p<0.001$). On the contrary, a significant decrease of HDL2 cholesterol and LDL cholesterol were observed in T2DM group compared to control group ($p<0.05$). The decrease of HDL2 cholesterol according to the cardiovascular risk factors was significant in obesity, diabetes duration up to 10 years and hypercholesterolemia. The HDL2 cholesterol variations according to diabetes complications showed significant decrease in metabolic syndrome and hypertension ($p<0.001$). The HDL2 cholesterol measurement was useful for evaluating cardiovascular risk in diabetics, because in this study only its level was significantly decreased, while total cholesterol and HDL3 cholesterol levels increased and LDL cholesterol level was within normal limits. **Conclusion:** Therefore, the HDL2 might be including in T2DM management. Indeed, the reagents are available from local providers and the quality control has validated this affordable method for resource limited laboratories.

Key words: Lipids, cholesterol subclasses, diabetes mellitus

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

According to the World Health Organization (WHO) projections, the number of deaths from diabetes in the world will double between 2005 and 2030 with Africa recording the highest increase in diabetes prevalence[1]. Currently, in Burkina Faso the prevalence of diabetes is between 3-6% in adult[2], similar to the prevalence in adults of 4.3% recorded in a developed country like France in 2009[3]. More than 50% of deaths from cardiovascular complications in diabetes have been reported by many prospective epidemiological studies making cardiovascular diseases the most reported cause of death in diabetic patients[4,5]. Hence, screening for lipid abnormalities should be an essential part of management of diabetic patients. It is recommended to perform an annual screening for blood fasting lipids, including Total Cholesterol (TC), triglycerides, High Density Lipoprotein Cholesterol (HDLC) and Low Density Lipoprotein Cholesterol (LDLC) in diabetic patients[6]. The HDLC has long been considered the main biological marker of protection against the cardiovascular disease[6]. However, many studies have reported cases of patients with low HDLC without additional risk of cardiovascular events and other increased risks, despite a high value of HDLC[7]. This atheroprotective role has been attributed to the HDL2 fraction of HDL cholesterol, differentiated on the basis of its density (d = 1,063-1,125 g mL^{-1}), whereas the HDL3 fraction (d = 1,1125-1,210 g mL^{-1}) does not seem involved[8,9]. Therefore, the HDL2 subfraction seem to be more atherosclerotic protective than the HDL3 subfraction[6]. A inverse correlation has been established between the HDL2 subfraction and atherosclerosis where an increase in the HDL2 subfraction results in decrease in its atherosclerosis[10]. Several methods of measurement of HDL subclasses have been proposed and many authors have demonstrated the value of knowledge of the HDL subclasses in the monitoring of metabolic diseases such as diabetes[11,12]. This has shown that there could be an association between HDL2 cholesterol and atherosclerosis in diabetic patients. This study was undertaken to further evaluate this association using the patients in Bukina Faso in order to build further to the body of knowledge in this area. This study also aims to validate a cost effective method for determining an effective dosage of HDL2C used in assessing cardiovascular risk. The authors propose that this will help Bukina Faso, a resource limited country, prevent coronary heart diseases.

MATERIALS AND METHODS

The study protocol and consent procedure were approved by the Burkina Faso National Ethics Committee for Research Ouagadougou, Burkina Faso approval No. 2012-06-52 on the 7th June, 2012. As required by the 1964 Helsinki declaration, a written informed consent was obtained from all participants prior to conducting any study procedures. After consenting, personal and epidemiological data were collected and recorded. All the data used in this study were anonymous.

Type and period of the study: This was a case-control study conducted between March to August, 2013 in Ouagadougou the capital city of Burkina Faso in West Africa. The Type 2 Diabetes Mellitus (T2DM) subjects were recruited at University Hospital Yalgado Ouedraogo of Ouagadougou and the control group at the Regional Center of Blood Transfusion of Ouagadougou. All laboratory tests were performed at the University Hospital laboratory Yalgado Ouedraogo of Ouagadougou.

Recruitment of study population: Included in the study were newly and previously diagnosed type 2 diabetes patients with Fasting Blood Glucose (FBG) >6.4 mmol L^{-1}. Subjects with or without complications of neuropathy, retinopathy, overt nephropathy, coronary artery diseases (hypertension or stroke) and attending the University Hospital Yalgado Ouedraogo of Ouagadougou (Burkina Faso) were included. Apparently healthy normotensive non-diabetic controls were recruited at the Regional Center of Blood Transfusion of Ouagadougou. Among type 2 diabetes patients, treatment with any lipid medication (statins, nicotinic acid, fibrate, resins) were excluded. Also, subjects should not have been enlisted in another concomitant study.

Biochemical analysis: After an overnight fast, venous blood was collected in a dry tube for biochemical analysis. Serum was separated by centrifugation at 3000 g for 10 min at 4°C, stored at -80°C and analyzed within a week. Serum Total Cholesterol (TC) and Triglycerides (TG) were determined using an automated Spintech 240 Biolis 24j analyzer (Spintech, Barcelona, Span) and the fully enzymatic methods (Spinreact kits Cholesterol-LQ reference TK41021 and Triglycerides-LQ reference TK41031). The dual-step precipitation of HDL subfractions was performed according to the procedure described by Hirano *et al.*[13]. To isolate total HDLC by precipitation, a combined precipitant consisting of 100 µL (0.02 mmol L^{-1}) of dextran sulfate (Mr 500000, SIGMA, France) and 25 µL (200 mmol L^{-1}) of $MnCl_2$ ($MgCl_2.6H_2O$, MERCK, France) was added to 1 mL of serum. After 15 min of standing at room temperature, the mixture was centrifuged at 3,400 g for 20 min at 4°C. Aliquots of the resulting supernatant (S1) were taken for the assay of the HDLC and precipitation of

the HDL2. The HDL2 was precipitated by a combined precipitant consisting of 100 µL (0.02 mmol L^{-1}) of dextran sulfate (Mr 500000, SIGMA, France) and 50 µL (200 mmol L^{-1}) of $MnCl_2$ ($MgCl_2.6H_2O$, MERCK, France) added to 500 µL of supernatant (S1). After 2 h at room temperature, the mixture was centrifuged at 3,400 g for 20 min at 4°C. Aliquots of the resulting Supernatant (S2) were taken for the assay of the HDL3C. The measured value for total HDLC was multiplied by 1.125 and that for HDL3 was multiplied by 2.92 to correct for dilution by the reagents. The HDL3C was measured by the direct HDLC homogenous assay instead of the original TC assay. The sub-fraction HDL2 was calculated by the following formula:

HDL2 cholesterol = HDL cholesterol-HDL3 cholesterol

The LDL cholesterol was calculated in mmol L^{-1} by using the Friedewald formula[14]:

LDL-C = TC-HDLC-TG/2.2

Quality control: To ensure the accuracy and precision of the test results, internal quality controls were performed daily before analysis and Standard Deviation (SD) and Coefficient of Variations (CV) calculated. Table 1 shows a summary of the quality control results with day to day coefficient of variations. The accuracy and precision of the measurements during the study in Table 1 were within the acceptable criteria stated in literature[15,16].

Statistical analysis: Quantitative variables were expressed as Means±SD and qualitative variables in percentages. The Analysis of Variance (ANOVA) was used to determine quantitative variables with normal distribution, followed by the Bonferonni multiple comparisons test to compare the means between groups. The statistical analysis was performed using the statistical software PASW, version 18 for Windows (SPSS CPSC., Chicago, USA). Probability levels of 0.05 or less were considered significant.

RESULTS

A total of 109 study subjects of which 66 (60%) were type 2 diabetes mellitus (T2DM) with an average age of 56±9 years were studied (Table 2). The other 43 (40%), making the control group, were non diabetic normotensive with an average age of 51±8 years. The 66 type 2 diabetic subjects, 24 (36.4%) and 42 (63.6%) were males and females, respectively.

Of the 43 in the control group, 24 (55.8%) were males and 19 (44.2%) females. There were significantly more females diabetics than males diabetics in the study group ($p<0.05$). The diabetics were significantly older than the controls (Control group mean = 51±8; T2DM group mean = 56±9; $p<0.05$). The male diabetics were older (mean = 57±8 years) than female (mean = 55±9 years). The age at which diabetes was diagnosed was 48.5±9.6 years and the median of the duration of having diagnosed diabetes was 6 years (Range = 0.5-27 years). The Body Mass Index (BMI) for diabetics (Mean = 28±5 kg m^{-2}) was significantly higher among diabetics ($p<0.001$) compared to non diabetics (Mean = 21.7±3 kg m^{-2}). The abdominal obesity frequency of type 2 diabetes group was 51.5% in female against 15.2% in male ($p<0.05$). The 37.9 and 36.4% of the T2DM group had microvascular and macrovascular complications, respectively. The main microvascular complications were retinopathy (57.1%), neuropathy (22.8%) and nephropathy (20%) while the main macrovascular ones were lower limb arteriopathy (64.5%) and angor (22.5%). The cardiovascular risks factors for type 2 diabetes (Fig. 1) were obesity (68%), abdominal obesity (66%), metabolic syndrome (54%), hypertension (50%), dyslipidemia (47%) and tabac (3%). Concerning the therapies, 80.3% were treated with oral antidiabetic, 10.6% treated with insulin, 6.1% treated with oral antidiabetic+insulin and 3% on dietetics regimen.

The total cholesterol, triglycerides, HDL cholesterol and HDL3 cholesterol (Table 3) were significantly higher in type 2 diabetes population compared to control group ($p<0.001$). On the contrary, a significant decrease of HDL2 cholesterol

Table 1: Accuracy and precision of the measurements and day to day coefficient of variations

	Accuracy (n = 20)			Precision (n = 20)	
Lipids	Reference mean	Mean obtained	Student t-test calculated	Intra-serial CV	Inter-serial CV
TC	2.87	2.81	0.83	0.56	0.98
HDLC	0.77	0.65	0.37	1.05	0.94
LDLC	1.54	1.39	1.67	0.98	1.56
TG	1.23	1.16	0.80	1.39	0.67

TC: Total cholesterol, HDLC: High density lipoprotein cholesterol, LDLC: Low density lipoprotein cholesterol, TG: Triglycerides, CV: Coefficient of variations

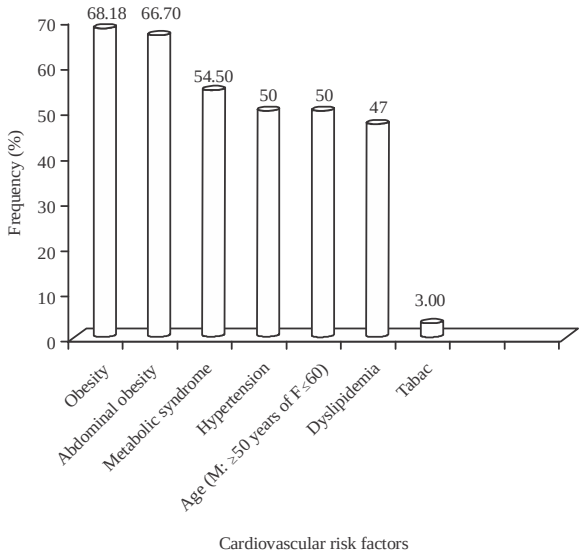

Fig. 1: Distribution of type 2 diabetes based on cardiovascular risk factors

Table 2: Demographic and clinical characteristics of the population of study

Demographic characteristics variables	Non diabetics n = 43 (M 24, F 19)	Type 2 diabetics n = 66 (M 24, F 42)
Age (years)		
Male (M)	54.0±9	57.0±8
Female (F)	48.0±7	55.0±9[a]
Total	51.0±8	56.0±9[a]
Sex ratio	1.3	0.57
Sex (No.) (%)		
Male	24.0 (55.8)	24.0 (36.4)
Female	19.0 (44.2)	42.0 (63.6)[b]
Age of diabetes discovery (years)	-	48.5±9.6
Duration of diabetes (years)		
Median	-	6.0
Extremes	-	0.5-27
BMI		
Male	20.0±3	26.1±5
Female	22.0±3	28.5±5
Total	21.0±3	28.0±5[a]
Abdominal obesity frequency (No.) (%)		
Male	-	10.0 (15.2)
Female	-	34.0 (51.5)[b]
Total	-	44.0 (66.7)
Microvascular complications (%)		
Retinopathy	-	57.1
Neuropathy	-	22.8
Nephropathy	-	20.0
Total	-	37.9
Macrovascular complications (%)		
Lower limb arteriopathy obliterans (LLAO)	-	64.5
Angor	-	22.5
Ischemic cardiopathy	-	6.4
Cerebral vascular accident (CVA)	-	3.2
Carotid atheroma	-	3.2
Total	-	36.4
Therapies (%)		
Oral antidiabetic	-	80.3
Insulin	-	10.6
Oral antidiabetic+insulin	-	6.1
Dietetics	-	3.0

Significant difference ($p<0.05$) between [a]Diabetics and non diabetics, [b]Males diabetics and females diabetics

Table 3: Blood lipids profile of study population

Lipids (mmol L^{-1})	Type 2 diabetics n = 66 (M 24, F 42)	Non diabetics n = 43 (M 24, F 19)	p-value
Total cholesterol			
Population	5.10±0.99	4.69±0.84	0.001[a]
Male	4.89±1.02	4.71±0.94	0.519
Female	5.16±0.98	4.67±0.72	0.001[e]
p-value	0.799	0.883	
Triglycerides			
Population	1.52±0.68	1.10±0.43	0.001[a]
Male	1.44±0.53	1.09±0.40	0.029[d]
Female	1.54±0,71	1.10±0.47	0.001[e]
p-value	0.666	0.965	
LDL			
Population	2.80±1.00	3.23±0.8	0.001[a]
Male	2.57±1.12	3.26±0.86	0.039[d]
Female	2.86±0.96	3.20±0.74	0.015[e]
p-value	0.671	0.821	
HDL			
Population	1.63±0.31	1.23±0.3	0.001[a]
Male	1.66±0.36	1.19±0.27	0.001[d]
Female	1.62±0.3	1.28±0.34	0.001[e]
p-value	0.241	0.331	
HDL3			
Population	1.22±0.29	0.76±0.18	0.001[a]
Male	1.21±0.37	0.76±0.20	0.001[d]
Female	1.22±0.27	0.76±0.15	0.001[e]
p-value	0.076	0.941	
HDL2			
Population	0.41±0.21	0.50±0.28	0.001[a]
Male	0.45±0.15	0.45±0.29	0.987
Female	0.40±0.22	0.56±0.28	0.001[e]
p-value	0.194	0.216	

Significant difference ($p<0.05$) between [a]Diabetics and non diabetics, [d]Males diabetics and males non diabetics, [e]Females diabetics and females non diabetics

and LDL cholesterol was observed in T2DM group compared to control group ($p<0.05$). The lipids levels between men and women within T2DM group and control group were not significantly different. However, the comparison by gender between type 2 diabetes group and control group showed significant differences ($p<0.001$) except in men for total cholesterol ($p = 0.519$) and HDL2 cholesterol ($p = 0.987$).

The decrease of HDL2 cholesterol according to the cardiovascular risk factors (Table 4) was significant in obesity group, diabetes duration up to 10 years group, all dyslipidemia group and hypercholesterolemia group ($p<0.05$). In women the decrease of HDL2 cholesterol was significant in abdominal obesity group, all dyslipidemia group, hypercholesterolemia group, mixed hyperlipidemia group and high cardiovascular risk group.

The HDL2 cholesterol variations according to diabetes complications in Table 5 showed significant decrease in metabolic syndrome and hypertension. In these complications (metabolic syndrome and hypertension) the HDL2 cholesterol decrease was significant only in women ($p<0.05$). In general, the HDL2 cholesterol was not significantly decreased according to cardiovascular risk factors and diabetes complications in men compare to control group.

DISCUSSION

Despite the limited resources, most laboratories in Bukina Faso are able to provide the lipoprotein's selective precipitation method, which in this study was validated by internal quality control, using dextran sulfate (MW 500,000) and magnesium chloride. Even though the reagents are available locally, making it more cost effective, existence of hypertriglyceridemia is an important limitation for this method. Hypertriglyceridemia makes incomplete precipitation of triglyceride-rich lipoproteins (VLDL and LDL)[15,17]. The analysis of sociodemographic and clinical characteristics of the type 2 diabetics reported a female predominance (63.6%), which could be explained by the observed increase in visitation by women in medical consultations in Burkina Faso.

The mean age of diagnosis of diabetes was 48±9 years and the median duration of diabetes was 6 years, ranging from 0.5-27 years. A mean duration was about 4 years in Ghana a neighboring country of Burkina Faso[18]. The duration of diabetes is known to be related to the development of complications[19]. This shows that the diagnosis of diabetes is late in Burkina at the time the cardiovascular complications are

Table 4: HDL2 variations according to cardiovascular risk factors

Groups	Non diabetics n = 43 (M 24, F 19)	Diabetes+ obesity BMI (>30) (M 16, F 26) n = 44	Diabetes+ age (M>50 F> 60) n = 33 (M 13, F 20)	Diabetes+ abdominal obesity n = 44 (M 10, F 34)	Duration of diabetes >10 years n = 21 (M 9, F 13)	Dyslipidemia Total n = 31 (M 10, F 21)	Hypertriglyceridemia n = 17 (M 7, F 10)	Hypercholesterolemia n = 20 (M 7, F 13)	Mixed hyperlipidemia n = 7 (M 2, F 5)	Diabetes+high cardiovascular risk factors
Population	0.50±0.28	0.26±0.18[a]	0.43±0.20	0.41±0.21	0.43±0.25[a]	0.34±0.17[a]	0.30±0.16[a]	0.36±0.16	0.31±0.14	0.42±0.19
Male	0.45±0.29	0.40±0.13	0.41±0.14	0.46±0.17	0.56±0.12	0.42±0.15	0.28±0.03	0.45±0.14	0.30±0.15	0.41±0.17
Female	0.56±0.28	0.37±0.12	0.44±0.23	0.39±0.21[e]	0.42±0.26	0.32±0.17[e]	0.30±0.18[e]	0.34±0.15	0.31±0.17[e]	0.42±0.19[e]
p-value	0.194	0.406	0.319	0.578	0.334	0.373	0.181	0.417	0.408	0.256

Significant difference (p<0.05) between [a]Diabetics and non diabetics, [e]Females diabetics and females non diabetics

Table 5: HDL2 variations according to diabetes complications

			Diabetes+macrovascular complications				Diabetes+microvascular complications		
Groups	Non diabetics n = 43 (M 24, F 19)	Metabolic syndrome n = 36 (M 16, F20)	All macrovascular complications n = 33 (M13, F 20)	LLAO n = 20 (M 7, F 13)	Angor n = 7 (M 2, F 5)	Hypertension n = 33 (M 14, F 19)	All microvascular complications n = 25 (M 10, F 15)	Nephropathy n = 7 (M 3, F 4)	Retinopathy n = 20 (M 7, F 13)
Population	0.50±0.28	0.39±0.20[a]	0.44±0.2	0.44±0.19	0.52±0.21	0.37±0.20[a]	0.43±0.23	0.48±0.24	0.45±0.28
Male	0.45±0.29	0.37±0.12	0.46±0.26	0.46±0.26	0.76±0.50	0.32±0.07	0.43±0.18	0.45±0.17	0.41±0.19
Female	0.56±0.28	0.40±0.22[e]	0.44±0.20	0.44±0.19	0.48±0.20	0.37±0.21[e]	0.43±0.25	0.51±0.24	0.44±0.23
p-value	0.194	0.231	0.321	0.274	0.396	0.354	0.357	0.335	0.434

LLAO: Lower limb arteriopathy obliterans, Significant difference (p<0.05) between [a]Diabetics and non diabetics, [e]Females diabetics and females non diabetics

already existing. Thus, in the study, over one third of type 2 diabetic patients had at least one macrovascular complication (36.4%) with lower limb arteriopathy obliterans being the main macrovascular complication (64.5%) followed by angor (22.5%). The prevalence of microvascular complications was 37.9% with retinopathy of 57.1%; neuropathy (22.8%) and nephropathy of (20%). For cardiovascular risk factors, 68% of type 2 diabetics were obese, more than half (54.5%) had metabolic syndrome, hypertension (50%) and dyslipidemia (47%). These findings are consistent with reports from other studies[18,20]. Indeed conventional lipid markers (total cholesterol and triglyceride) were significantly increased, confirming high cardiovascular risk in diabetics. The mean TC level for diabetics was significantly higher than for non diabetics ($p<0.001$) but no significant difference was observed between male diabetics and male controls. The T2DM total cholesterol level remained in the normal range. Studies have recorded lower total cholesterol level in diabetes[2,21]. This could be explained by the fact that in type 2 diabetes mainly LDL cholesterol qualitative abnormalities conversely quantitative abnormalities for HDL cholesterol were reported[22]. The mean TG level for diabetics was also significantly higher compared to non diabetics ($p<0.001$). Indeed, VLDL production increases as the substrates (fatty acids and glucose) increases in diabetes and a delayed catabolism due to a reduced activity of the lipoprotein lipase, which allows the hydrolysis of the low density particles (VLDL and chylomicrons). In this study, the LDL cholesterol level was significantly decreased in diabetics compared to the control group. Ruderman and Haudenschild[23] also reported low-density lipoprotein cholesterol to be normal in diabetics. In type 2 diabetes, there appears to be no significant increase in LDL, but rather qualitative abnormalities[22]. The LDL cholesterol in type 2 diabetes is small dense compare to normal LDL. These small dense LDL plays a central role in atherogenesis and is associated with an increased risk of cardiovascular disease[10,24].

Of note, in this study the HDL cholesterol was significantly increased in diabetic patients compared to controls. The HDL cholesterol increase can be explained by the increase of the HDL3 cholesterol fraction in the diabetics group. The abnormally high levels of insulin in diabetics leads to hyper-activation of hepatic lipase which converts HDL2 cholesterol into HDL3 cholesterol[25]. For that reason a statistically significant decrease in HDL2 cholesterol in T2DM patients compared to control was observed. This decrease in HDL2 cholesterol is explained by the fact that the apoAI, major protein component of HDL2, is reduced in type 2 diabetes[26]. The decrease of HDL2 cholesterol according to the cardiovascular risk factors was significant in obesity group, diabetes duration up to 10 years group, all dyslipidemia group and hypercholesterolemia group. In women the decrease of HDL2 cholesterol was significant in abdominal obesity group, all dyslipidemia group, hypercholesterolemia group, mixed hyperlipidemia group and high cardiovascular risk group. These findings are consistent with reports from other studies[8,9,18]. The HDL2 cholesterol variations according to diabetes complications showed significant decrease in metabolic syndrome and hypertension. The dyslipidemia seen in the T2DM patients with hypertension could be due to the effects of treatment. In other studies, treatment of hypertension with b-blockers, as well as high doses of thiazide diuretics have been shown to exacerbate the dyslipidemia in patients with hypertension and diabetes mellitus[27,28]. Regarding the HDL2 decrease in metabolic syndrome in this study, recent data have suggested a relationship between the HDL2/HDL3 ratio and metabolic syndrome. These data indicated that the HDL2/HDL3 ratio was associated with insulin resistance and was gradually decreased as the number of metabolic syndrome components increased[29].

CONCLUSION

The HDL2 cholesterol measurement was useful for evaluating cardiovascular risks in diabetics because as shown by this study that only its level was significantly decreased, while total cholesterol and HDL3 cholesterol levels increased and LDL cholesterol level were within normal limits. Therefore, HDL2 might be a better predictor of cardiovascular diseases than total HDL in diabetes mellitus. Moreover, the reagents are available locally and this method was validated by internal quality controls, making this cost effective for a country of limited resources.

REFERENCES

1. Wild, S., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care, 27: 1047-1053.
2. Sagna, Y., H. Tieno, O. Guira, D.A.R. Yanogo and L.E. Benon *et al*., 2014. Prevalence and associated risk factors of diabetes and impaired fasting glucose in urban population; a study from Burkina Faso. J. Diabetol., Vol. 2.
3. Mandereau-Bruno, L., P. Denis, A. Fagot-Campagna and S. Fosse-Edorh, 2014. [Prevalence of people pharmacologically treated for diabetes and territorial variations in France in 2012]. Bulletin Epidemiologique Hebdomadaire, 30-31: 493-499, (In French).

4. Anderson, J.L., C.D. Adams, E.M. Antman, C.R. Bridges and R.M. Califf *et al*., 2007. ACC/AHA 2007 guidelines for the management of patients with unstable angina/ non-ST-elevation myocardial infarction. J. Am. Coll. Cardiol., 7: e1-e157.
5. Malati, T. and M.R.U. Mahesh, 2009. Reference intervals for serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, Lp (a), apolipoprotein A-I, A-II, B, C-II, C-III and E in healthy South Indians from Andhra Pradesh. Indian J. Clin. Biochem., 24: 343-355.
6. McPherson, R., J. Frohlich, G. Fodor and J. Genest, 2006. Canadian cardiovascular society position statement-recommendations for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease. Can. J. Cardiol., 22: 913-927.
7. Joy, T. and R.A. Hegele, 2008. Is raising HDL a futile strategy for atheroprotection?. Nat. Rev. Drug Discov., 7: 143-155.
8. Bakogianni, M.C., C.A. Kalofoutis, K.I. Skenderi and A.T. Kalofoutis, 2001. Clinical evaluation of plasma high-density lipoprotein subfractions (HDL2, HDL3) in non-insulin-dependent diabetics with coronary artery disease. J. Diabetes Complic., 15: 265-269.
9. Moriyama, K., M. Negami and E. Takahashi, 2014. HDL2-cholesterol/HDL3-cholesterol ratio was associated with insulin resistance, high-molecular-weight adiponectin and components for metabolic syndrome in Japanese. Diabetes Res. Clin. Pract., 106: 360-365.
10. Maeda, S., S. Nakanishi, M. Yoneda, T. Awaya, K. Yamane, T. Hirano and N. Kohno, 2012. Associations between small dense LDL, HDL subfractions (HDL2, HDL3) and risk of atherosclerosis in Japanese-Americans. J. Atheroscler. Thromb., 19: 444-452.
11. Superko, H.R., 2009. Advanced lipoprotein testing and Subfractionation are clinically useful. Circulation, 119: 2383-2395.
12. Shuhei, N., S. Soderlund, M. Jauhiainen and M.R. Taskinen, 2010. Effect of HDL composition and particle size on the resistance of HDL to the oxidation. Lipids Health Dis., Vol. 9 10.1186/1476-511X-9-104
13. Hirano, T., K. Nohtomi, S. Koba, A. Muroi and Y. Ito, 2008. A simple and precise method for measuring HDL-cholesterol subfractions by a single precipitation followed by homogenous HDL-cholesterol assay. J. Lipid Res., 49: 1130-1136.
14. Srisawasdi, P., S. Chaloeysup, Y. Teerajetgul, A. Pocathikorn, C. Sukasem, S. Vanavanan and M.H. Kroll, 2011. Estimation of plasma small dense LDL cholesterol from classic lipid measures. Am. J. Clin. Pathol., 136: 20-29.
15. Talameh, Y., R. Wei and H. Naito, 1986. Measurement of total HDL, HDL_2 and HDL_3 by dextran sulfate-$MgCl_2$ precipitation technique in human serum. Clinica Chimica Acta, 158: 33-41.
16. Vassault, A., D. Grafmeyer, J. de Graeve, R. Cohen, A. Beaudonnet and J. Bienvenu, 1999. Analyse de biologie medicale: Specification et normes d'acceptabilite a l'usage de la validation de techniques. Ann. Biol. Clin., 57: 685-695.
17. Dias, V.C., H.G. Parsons, N.D. Boyd and P. Keane, 1988. Dual-precipitation method evaluated for determination of high-density lipoprotein (HDL), HDL2 and HDL3 cholesterol concentrations. Clin. Chem., 34: 2322-2327.
18. Adinortey, M.B., B.E. Gyan, J. Adjimani, P. Nyarko, C. Sarpong, F.Y. Tsikata and A.K. Nyarko, 2011. Dyslipidaemia associated with type 2 diabetics with micro and macrovascular complications among Ghanaians. Indian J. Clin. Biochem., 26: 261-268.
19. Davis, M.D., 1992. Diabetic retinopathy: A clinical overview. Diabetes Care, 15: 1844-1874.
20. Mullugeta, Y., R. Chawla, T. Kebede and Y. Worku, 2012. Dyslipidemia associated with poor glycemic control in type 2 diabetes mellitus and the protective effect of metformin supplementation. Indian J. Clin. Biochem., 27: 363-369.
21. Nyarko, A., J. Asiedu-Larbi, M. Ofosuhene, H. Asare-Anane and M.E. Addy, 2003. Serum lipids and antioxidants in Ghanaian diabetic, hypertensive and healthy subjects. Ghana Med. J., 37: 72-82.
22. Boden, G., 2001. Pathogenesis of type 2 diabetes: Insulin resistance. Endocrinol. Metabol. Clin. North Am., 30: 801-815.
23. Ruderman, N.B. and C. Haudenschild, 1984. Diabetes as an atherogenic factor. Progr. Cardiovasc. Dis., 26: 373-412.
24. Packard, C., M. Caslake and J. Shepherd, 2000. The role of small, dense Low Density Lipoprotein (LDL): A new look. Int. J. Cardiol., 74: S17-S22.
25. Wang, H. and D.Q. Peng, 2011. New insights into the mechanism of low high-density lipoprotein cholesterol in obesity. Lipids Health Dis., Vol. 10. 10.1186/1476-511X-10-176
26. Van Linthout, S., F. Spillmann, H.P. Schultheiss and C. Tschope, 2010. High-density lipoprotein at the interface of type 2 diabetes mellitus and cardiovascular disorders. Curr. Pharmaceut. Des., 16: 1504-1516.
27. Andrew, J.K. and J.B. Clifford, 1994. Type 2 Diabetes. Royal Society of Medicine Press, London, Pages: 107.
28. Iaccarino, G., V. Trimarco, F. Lanni, E. Cipolletta and R. Izzo *et al*., 2005. β-Blockade and increased dyslipidemia in patients bearing Glu27 variant of β_2 adrenergic receptor gene. Pharmacogenomics J., 5: 292-297.
29. Paavola, T., S.M. Kuusisto, T. Kangas-Kontio, J. Metso and M. Jauhiainen *et al*., 2014. Impaired cholesterol efflux into hdl2 in metabolic syndrome. Atherosclerosis, 235: e183-e183.