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Detection of lipoprotein(a)- cholesterol expression in Bangladeshi adults with dyslipidemia

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ABSTRACT

Lipoprotein(a)-cholesterol (Lp(a)-C), a low-density lipoprotein (LDL)-like particle is considered as a risk factor for cardiovascular diseases (CVDs). We aimed to investigate the association of Lp(a)-C expression with dyslipidemia among the Bangladeshi population and assess the relationship with cardiovascular risks. In this cross-sectional comparative study, a total of 180 urban males and females between ages 19-65 years were included who were enrolled in a hospital setting of Bangladesh. Participants were selected based on their total cholesterol (TC) level≥200 mg/dl, high density lipoprotein (HDL)-C<40 mg/dl, LDL-C≥140 mg/dl, and triacylglycerol (TG)≥150 mg/dl regardless of race, religion and socioeconomic status. Venous blood was collected from all participants and analyzed. Further, participants' socio-demographics and body mass index (BMI) were collected. Expression of Lp(a)-C was detected in 22.86% patients with desirable levels (<14 mg/dL) of serum Lp(a)-C. This study suggests that the prevalence of hyperlipidemia and hypertriglyceridemia is high in the Bangladeshi population. Males were found to have lower HDL-C and higher TG than females. and, similar to other ethnic groups, a negative correlation between BMI and HDL-C was found in this population. In addition, Lp(a)-C had a positive correlation with TG which may recommend routine clinical investigation of Lp(a)-C as a biomarker for CVD risk.

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1. INTRODUCTION

Dyslipidemia refers to an abnormal amount of lipids or fats carried by lipoproteins in the blood [1]. According to WHO, dyslipidemia is a highly prevalent disorder (38.9%) in the worldwide [2] that is associated with decreased longevity and increased morbidity resulting from a wide range of pathological conditions, including insulin resistance, obesity, hypertension, cardiovascular diseases (CVDs), and atherosclerosis [3]. The lipids that are commonly measured in blood include various forms of cholesterol, such as high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TGs) as well as total cholesterol (TC). Though lipids are essential to life, an abnormal amount of certain lipids can increase the risks for CVDs [4-5]. For example, HDL-C is known as the "good cholesterol," but its lower level may increase the risk of CVDs. On the other hand, LDL-C, known as the "bad cholesterol," is linked to increased risk of heart attacks and strokes [6].

Lipoprotein(a)-cholesterol (Lp(a)-C) is an LDL-like particle in which apolipoprotein(a), a glycoprotein consisting of several repetitive kringle IV type 2 (KIV-2) structures and homology to plasminogen [7], is attached to apolipoprotein B by a disulphide linkage [8]. High levels of Lp(a)-C in blood

can create plaque, a buildup of cholesterol that limits blood flow through arteries. Elevated concentrations of Lp(a)-C has been identified as the strongest risk factor for CVDs in the general population [9]. Variations in the levels of Lp(a)-C are mainly genetically determined [10] in which the level of Lp(a)-C is inversely correlated with the number of KIV-2 [11]. Lp(a)-C concentration can also vary from population to population [12-13]. Other factors such as diet, drugs, hormones and glycemic control are also found to affect Lp(a)-C concentration [14]. Previous in vitro, animal, and epidemiological studies suggest controversial results that Lp(a)-C may contribute to the development of atherosclerosis or thrombosis and thus increase the frequencies of myocardial infarction and ischemic heart diseases [15-18].

According to the world health report in 2003, CVDs were responsible for more than 80% of deaths in South Asia, which is 40-60% higher than any other population [19]. Different studies have found improvement from CVDs by lowering the cholesterol levels [20-21]. However, most of the dyslipidemic studies in Bangladesh are focused on the relationship with diabetes [22-24] and sufficient data is not available for the assessment of cardiovascular risks with dyslipidemia. This study therefore aims to investigate the expression of Lp(a)-C in Bangladeshi dyslipidemic patients and their relation to the risks of CVDs. To the best of our knowledge, there is lack of evidence on expression of Lp(a)-C levels among the Bangladeshi population.

2. RESEARCH METHOD

2.1. Study design and participants

We conducted a cross-sectional comparative study among 180 males and females from July 2015 to June 2016. Of these subjects, 140 males and females were dyslipidemic and 40 males and females were non-dyslipidemic, which served as the comparison group. We selected urban residing population aged 19-65 years. The study was carried out in a hospital setting, approximately 24 km away from Dhaka-capital of Bangladesh. Dyslipidemic subjects with cholesterol≥200 mg/dl, HDL-C<40 mg/dl, LDL-C≥140 mg/dl, and TG≥150 mg/dl were recruited in this study irrespective of race, religion and socioeconomic status. Hyperlipidemia (high levels of TC and TG), hypertriglyceridemia (high levels of TG), atherogenic dyslipidemia (high levels of TG, LDL-C, low levels of HDL-C) and hypercholesterolemia (high levels of TC) were considered as different types of dyslipidemia.

2.2. Variable assessed

Data on age (in years), gender (male/female), physical exercise (yes/no), dietary habit (consumption of red meat three times or more per week: yes/no), smoking (yes/no), history of diabetes mellitus (yes/no), and hypertension (HTN: yes/no) were collected by interviewing participants. Physical exercise was defined as at least 30 minutes of planned physical movement to improve one's fitness [25]. Individuals having more than five cigarettes per day was considered a positive smoking condition [26]. Valid cases of diabetes mellitus and HTN were considered by those who have been earlier diagnosed by a registered physician and provided the prescription. Body weight of the subjects was measured (to the nearest 0.1 Kg) by standing motionless on a digital weighing machine. Height was measured (to the nearest 0.1 cm) by Leicester Height Measure. Body Mass Index (BMI) (expressed in kg/m^2) was calculated and categorized as follows: underweight<18.5, normal weight 18.5-24.9, overweight 25-29.9; and obese>30 [27]. According to the guideline of the Joint National Commission on Prevention, Detection, Evaluation, and Treatment of High Blood pressure (BP) (JNC 7), participant's systolic blood pressure (SBP)≥140 mmHg and/or diastolic blood pressure (DBP)≥90 mmHg was considered as HTN [28]. BP was measured by using a digital blood pressure monitor machine (©OMRON Healthcare Europe B.V) after the subject was rested for 10 minutes in the sitting position. We repeated the BP measurements three times in 10 minutes interval.

2.3. Collection of blood samples

Overnight fasting (8-10 hours) venous blood was collected between 8:00-9:00 am. Venous blood (5 ml) was obtained by the hospital phenologist using venipuncture following the standard procedure. The blood was taken in a plain tube and allowed to clot for 30 minutes. Serum was separated by centrifugation for 10 min at 3000 rpm using a refrigerated centrifuge and used for further biochemical analyses. The lipid profiles (TC, HDL-C, LDL-C, TG) were measured using an Automatic Analyzer (QCA mini Discrete Random- Access Analyzer, Spain).

2.4. Estimation of Lp(a)-C

Overnight fasting (8-12 hours) blood samples were taken in a plain tube and allowed to clot for 30 minutes. Serum was separated by centrifugation to detect the expression of Lp(a)-C using the Helena

BioSciences SAS-1 Cholesterol Profile-12 Kit (Helena BioSciences, UK) Manufacturer provided protocol was used for gel electrophoresis. The SAS-1 Cholesterol Profile-12 Kit separates serum lipoproteins according to charge in a buffered agarose gel. The alpha band, which migrates fastest towards the anode, corresponds to HDL. The next band, prebeta, corresponds to VLDL and the slowest moving beta band corresponds approximately to LDL. A band may appear between the alpha and pre-beta bands on some, but not all, samples-this should be quantitated as the Lp(a)-C fraction. Gels were scanned using a 570nm filter and expression levels of Lp(a)-C were analyzed densitometrically using platinum.exe software.

2.5. Statistical analysis

Both descriptive and inferential statistics were used in this study. Continuous variables were presented as mean±standard deviation (SD) and the statistical difference between groups was assessed by Independent Sample's T-test and Analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post hoc. Paired samples correlation was performed to calculate measure correlation. Significance level was set at <0.05. Statistical Package for Social Sciences (SPSS) software, version 13 was used.

3. RESULTS AND DISCUSSIONS

In Bangladesh, though most of the studies on dyslipidemia investigate the different levels of lipid components, the pattern of dyslipidemia and Lp(a)-C expression in dyslipidemic subjects has not specifically been studied. In this study, we focus on the pattern of dyslipidemia associated with compounding risk factors like age, gender, BMI, hypertension, which can complicate the risk of CVDs. Also, the expression of Lp(a)-C in dyslipidemic subjects was detected to associate with CVDs. Based on the laboratory findings of abnormal lipid profiles of the out patients, dyslipidemic subjects were included in this study.

To evaluate dyslipidemia as a risk factor for CVDs in Bangladeshi population, at first, we examined the BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the study subjects. Dyslipidemic patients had significantly higher BMI, SBP and DBP compared to non-dyslipidemic patients as shown in Table 1. This indicates that dyslipidemic patients are at a higher risk for CVD events than non-dyslipidemic subjects. Moreover, the level of HDL-C was significantly lower which is known as one of the risk factors for CVDs [29-30] and TG was significantly higher in dyslipidemic subjects compared to non-dyslipidemic subjects.

Table 1. Variation in BMI, BP and lipid profiles between dyslipidemic and non-dyslipidemic group

Variables	Mean±(SD)		P value*†
	Dyslipidemic (N 140)	Non-dyslipidemic (N 40)	
BMI (kg/m2)	25.74±4.57	23.39±3.57	0.004*
Blood pressure			
SBP (mmHg)	137.11±15.82	115.57±8.9	0.000*
DBP (mmHg)	88.82±11.25	73.81±7.08	0.000*
Lipid components			
TC (mg/dL)	199.78±44.50	184.75±36.24	0.052
HDL-C (mg/dL)	35.98±10.25	44.70±12.85	0.000*
LDL-C (mg/dL)	117.07±37.46	110.33±35.21	0.311
TG (mg/dL)	235.08±166.1	148.58±74	0.000*

†*Independent Sample's t-test*
**p=<0.05*

The differences between genders in individuals with dyslipidemia and status of individual lipid components are presented in Table 2. The level of HDL-C was significantly lower in males compared to their counterpart, whereas the TG is significantly higher in males compared to females. This indicates that males are more prone to becoming dyslipidemic and are therefore at a high risk for CVDs [31]. In addition, researchers found an inverse correlation between smoking and HDL-C level [32]. In our study, most of the males were smokers and had low levels of HDL-C. This pattern is also a risk factor for CVDs therefore our study suggests that CVDs are more prevalent in the male population.

We noted that a considerable number of middle-aged populations (40-50 years) had been suffering from dyslipidemia compared to the young and old population; however, we did not find significant differences in different age groups. Although we found insignificant differences in the serum levels of the lipid components among the different age groups, the TG levels were higher in older ages. We noted a higher number of dyslipidemic patients with elevated blood pressure, although the numbers were not significantly different. However, a previous study has shown a positive relation between HTN and abnormal dyslipidemia

as well as CVDs [33]. In regard to BMI, we found that most of the dyslipidemic subjects were overweight. Moreover, most of the dyslipidemic males were overweight, whereas the majority of the females' BMI were normal.

Correlation of BMI of the dyslipidemic subjects with serum levels of individual lipid components was also analyzed. From our data, correlations of BMI with TC, LDL-C and TG were all statistically insignificant. However, negative correlation of BMI with HDL-C in the studied Bangladeshi population was highly remarkable (p=0.05) which is similar to other ethnic groups [33]. From this point of view, we can explain that with the increasing of BMI, good cholesterol (HDL-C) is decreasing which is associated with CVD risk. Advancing age, high levels of BMI, SBP, DBP and abnormal lipid profiles are considered risk factors for CVDs in both genders [34-35]. In our study, dyslipidemic subjects are more aged and have high BMI, SBP and DBP than that of normal subjects and therefore increase the risk of CVDs in their life.

Table 2. Comparison of lipid profile based on gender, age, BP and BMI among dyslipidemic participants

Variables	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
Gender				
Male	193±44	32.9±8	110.5±38	268±181.22
Female	207±44	40±10.5	124.6±35.6	197.16±138.5
P value[*†]	0.064	0.000*	0.026*	0.011*
Age				
19-39 years	199.5±38	36.2±9.4	118±32	212.5±105
40-50 years	202.6±43.7	36.9±10	118.2±35.8	240.8±180
51-65 years	196.2±51.3	35.3±10.3	114.7±44.4	248±192
P value[*»]	0.778	0.739	0.883	0.586
Blood pressure				
Normotensive	194.05±36.6	35.85±8.71	116.91±29.98	202.97±146.28
Hypertensive	202.77±48.02	36.44±10.49	117.16±40.98	251.83±173.88
P value[*†]	0.273	0.738	0.970	0.083
Correlation with BMI *(r/'p' value)*[#]	0.057/0.502	-0.166/0.050	0.069/0.416	0.011/0.901

†Independent Sample's t-test
»ANOVA followed by Fisher's least significant difference (LSD) post hoc
Paired samples correlation
*$p=<0.05$

Next, it is detected the expression level of Lp(a)-C by agarose gel electrophoresis using the Helena BioSciences SAS-1 Cholesterol Profile Kit (Helena BioSciences, UK). Expression of Lp(a)-C was detected among 22.9% (n=32) of 140 dyslipidemic subjects in this study (data not shown). Among them, 31.25% were female and 68.75% were male. The mean Lp(a) level was 9.36±6 mg/dL (Data not shown). The reference values for Lp(a)-C are: Desirable<14 mg/dL (<35 nmol/L), Borderline risk 14-30 mg/dL (35-75 nmol/L), High risk 31-50 mg/dL (75-125 nmol/L), and very high risk>50 mg/dL (>125 nmol/L) [36-37]. From this reference, we found desirable Lp(a)-C levels in our studied population.

Most of the Lp(a)-C containing dyslipidemic subjects were above 40 years. As shown in Table 3, differences in the level of lipid components among the different age groups were insignificant but the levels of TC, TG and LDL-C were relatively higher in the 40-50 years age group. The HDL-C levels in serum were significantly higher in the females compared to the males. Although statistically insignificant (p=0.277), serum levels of TG in male subjects were higher than that of female subjects. In terms of TC, HDL-C, LDL-C, no significant correlation was found with Lp(a)-C level. However, there was significant positive correlation between TG and Lp(a)-C level (r=0.423, p=0.016).

Lp(a)-C is thought as an independent risk factor for CVDs, and thereby association between the level of Lp(a) and TG is controversial [38-39]. Though the number of Lp(a)-C containing subjects in our study was low, based on our findings it may be a notable risk factor for CVDs in this population. Moreover, the positive correlation between Lp(a)-C and TG levels may recommend routine clinical investigation of Lp(a)-C as a biomarker for CVD risk.

We also investigated different habits including smoking, exercise and red meat consumption among in dyslipidemic subjects and also in the subjects with Lp(a)-C expression to find out the relationship of those habits with the risk of development of CVDs as shown in Table 4. A large number of dyslipidemic subjects had higher smoking history and more consumption of red meat as shown in Table 4. Interestingly, Lp(a)-C expression levels were also found to be associated with living style. Previous report showed that smoking lowers the level of HDL-C [32]. Though diet has a lesser effect on Lp(a)-C, different studies showed that pecan, walnuts and fish oil can lower the level of Lp(a)-C [39-41]. Moreover, previous findings also supported a direct association between consumption of red meat and

CVDs [42-43]. As red meat increases the level of plasma TG [44], it may increase the risk of CVDs in subjects with Lp(a)-C.

Table 3. Lipidemic status of dyslipidemic subjects with Lp(a)-C expression

Variables	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
		Gender		
Male	190±37.6	33.35±6.4	106.15±35.31	268.5±108.5
Female	196±46.7	42±12.2	107.5±40.2	226±79
P value*†	0.697	0.011*	0.921	0.277
		Age		
19-39 years	201.1±35	35±10	117.7±31	220.5±65
40-50 years	192.8±39	35.2±8.8	104±38.1	288.2±114.8
51-65 years	183±47.4	38.6±10.7	102.7±38.8	223.5±84.8
P value*»	0.677	0.655	0.669	0.183
Correlation Between Lp(a)-C and Lipid Profile (r/'p' value)*#	0.032/0.864	0.135/0.461	(-)0.156/0.394	0.423/0.016*

†Independent Sample's t-test
»ANOVA followed by Fisher's least significant difference (LSD) post hoc
#Paired samples correlation
*$p=<0.05$

Table 4. Demographic distribution of the study participants

Factor	Habit	Dyslipidemic subjects (n=140)	Lp(a)-C subjects (n=32)
Smoking	No	32.9% (46)	34.4% (11)
	Yes	67.1% (94)	65.6% (21)
Exercise	No	50% (70)	40.6% (13)
	Yes	50% (70)	59.4% (19)
Red Meat	No	47.1% (66)	43.8% (14)
	Yes	52.9% (74)	56.3% (18)

Frequency is expressed as %(N)

CVDs are major health problems throughout the world and a common cause of premature morbidity and mortality in South Asia [19]. Early diagnosis of dyslipidemia can decrease the probability of morbidity and mortality caused by CVDs. However, Lp(a)-C remains an enigmatic lipoprotein though it had been identified more than 50 years ago by Kare Berg [45], and prior to the discovery of the genetic sequence of apo(a) over 20 years ago by McLean et al [7]. Although a risk factor role of Lp(a)-C for CVDs has been controversial, recent studies have supported the role of Lp(a)-C in promoting CVDs. In our study, both male and female dyslipidemic subjects with Lp(a)-C expression commonly displayed hyperlipidemia and hypertriglyceridemia. Although statistically insignificant, serum level of TG in male subjects was higher than that of the female subjects, and TC was higher in females compared to males. Most of the Lp(a)-C expressing subjects were overweight and in age between 40-50 years. The TG level in this age was also high. Most of the dyslipidemic subjects with Lp(a)-C expression had hypertension which is similar to other studies [46-47]. The positive correlation between Lp(a)-C and TG level can be explained by the association of Lp(a)-C containing particle apo(a) with plasma TG [48]. These findings indicate that dyslipidemic subjects with Lp(a)-C expression are more prone to experience CVDs in their life. The strength of this study includes direct collection of blood and analysis of the samples. Despite the strength, the study has a couple of limitations. One limitation of our study was that we did not measure the Lp(a)-C level of the control subjects in order to compare it to the Lp(a)-C level in dyslipidemic subjects. This gap can be included in future work and more research is needed to investigate the prevalence of Lp(a)-C in a wide range of the Bangladeshi population. Another limitation is the disproportionate number of individuals in the comparison and target groups (140 vs 40), however it would unlikely affect the results as we have analyzed the biological samples and reported biological variations of both groups.

4. CONCLUSION

The research found that the prevalence of hyperlipidemia and hypertriglyceridemia is high in the Bangladeshi population and most of the Lp(a)-C expression is found in the dyslipidemic group, which may be considered as a risk factor for CVDs.

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