

# Apo B/Apo A-I Ratio is Statistically A Better Predictor of Cardiovascular Disease (CVD) than Conventional Lipid Profile: A Study from Kathmandu Valley, Nepal

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## ABSTRACT

**Background:** Apo B and Apo A-I, are structural and functional components of lipoprotein particles that serve as transporters of cholesterol. The apo B/apo A-I ratio reflects the cholesterol transport and has been shown to be strongly related to risk of Myocardial infarction, stroke and other Cardiovascular manifestations.

**Materials and Methods:** Forty five participants with Cardiovascular Disease (CVD) and forty four healthy participants were included from different locations of Kathmandu valley, Nepal. Fasting blood samples were collected from ante-cubital vein and

serum samples were used for lipid parameters, apo B and apo A-I levels measurement.

**Results:** Statistically significant differences were found for apo B/apo A-I ratio, HDL-c and apo B between the groups. The other lipid parameters and lipid ratios such as total cholesterol, triglyceride, low density lipoprotein, TC/HDL-c, TG/HDL-c and LDL-c/HDL-c were not found to be significant.

**Conclusion:** Apo B/apo A-I ratio seems to have better predictive value than that of classical lipid parameters in cardiovascular risk assessment.

**Keywords:** Apo B/apo A-I ratio, Cardiovascular disease, Lipid parameters, Predictive value

## INTRODUCTION

Over the years CVD has been the major threat to the global health. CVD attributed to 30% of the estimated 58 million deaths globally in 2005 [1]. More importantly, CVD is accounted for 79% of the disease burden in the productive period of life [2]. Non-communicable diseases are responsible for half of the disease burden in low and middle-income countries [3].

Dyslipidemia is one of the crucial cardiovascular risk factors for the progression of atherosclerosis leading to cardiovascular associated diseases [4-6]. Of the different apolipoproteins, apolipoprotein A-I (apo A-I) is associated with cardioprotective lipid (HDL-C) [7] while apolipoprotein B (Apo B) is associated with atherogenic lipids (LDL and VLDL) [8]. Apo B and apo A-I are structural and functional components of lipoprotein particles that serve as transporters of cholesterol. Apo B transfers cholesterol and triglycerides (TG) from site of production to tissues, where they are utilized for energy production, storage membrane assembly or hormone synthesis. Apo A-I plays major role in the reverse cholesterol transport by transferring cholesterol from tissue, back to the liver [7].

Very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), large buoyant LDL and small dense LDL (sd-LDL), all these atherogenic particles consist of apo B. Hence, total apo B represents the total amount of atherogenic particles. In addition, apo B is associated with entrapment of these lipoproteins in arterial wall and it also facilitates uptake of cholesterol in peripheral tissue and liver [9-10]. Unlike apo B, apo A-I is a major apolipoprotein of HDL particles which helps the reversal transport of cholesterol from peripheral tissue to liver, thus reducing the risk of developing inflammatory response and growth of plaques [11]. Nowadays, apo B and apo A-I can be measured directly by employing standardized and internationally validated techniques [12]. The apo B/apo A-I ratio reflects the cholesterol transport [13] and has been shown to be strongly related to risk of myocardial infarction, stroke and other

cardiovascular manifestations [14].

The change in lifestyle trend and increasing CVD burden in a country like Nepal led us to devise this study to evaluate the predictive value of apo B/ apo A-I ratio.

## MATERIALS AND METHODS

The study consists of 89 participants, 45 patients with CVD (cases) and 44 healthy participants (controls) aged > 30 years. The study was carried out from September 2012 to January 2013. Patients with CVD such as coronary artery disease (CAD), coronary heart disease (CHD), myocardial infarction (MI) and ischemic heart disease (IHD) were considered for our study after the confirmative diagnosis done by the cardiologist based on the findings of electrocardiography (ECG), electrocardiogram (EKG), raised cardiac enzymes (creatine kinase-MB, lactate dehydrogenase (LDH), serum glutarate pyruvate transaminase (SGPT), serum glutarate oxaloacetate transaminase (SGOT) and troponin I. Controls were investigated for history of diabetes, hormonal disorders, liver diseases, renal diseases and other chronic diseases. Those suspected of any disorders as indicated above were excluded. Ethical clearance was taken from concerned authority and consent from individual participants.

### Sample Collection

After an overnight fast of 12 hour, blood samples were collected from the ante-cubital vein of each participant. Samples were collected in plain vials, allowed to clot, centrifuged at 3,000 rpm; 10 minutes and serum separated and preserved at -20° C until assays were run.

### Biochemical Analysis

The lipid profile was done using CHOD-PAP method (Systemic Reagent for Humastar 600, Human, Germany). The LDL was

calculated using the Friedewald formula [15]. All the biochemical tests were run in the fully autoanalyzer (Humastar 600, Human, Germany). The serodos and serodos plus were used as the quality control samples and autocal as the standard to calibrate the tests. Both, internal and external, quality assurance tools were employed routinely to ensure the quality of test results.

The apo B and apo A-I concentrations were measured using immunoturbidimetric method (Systemic Reagent for Humastar 600, Human, Germany).

## STATISTICAL ANALYSIS

All the statistical analysis were done using IBM SPSS Statistics (version 19) software. All tests of statistical significance were two-sided with 95% Confidence Intervals (CI).

## RESULTS

The demographics of the study population is given in [Table/Fig-1]. Significant differences in apo B/apo A-I ratio ( $p=0.026$ ), HDL-c ( $p=0.007$ ) and apo B ( $p=0.020$ ) were observed between the groups [Table/Fig-2]. The lipid profile including TC, TG, LDL, nonHDL-c and the lipid ratios TC/HDL-c, TG/HDL-c and LDL/HDL-c were found to be statistically insignificant. Similarly apo A-I showed no significant difference.

Sex	Cases	Controls	Total
Male	24 (53.3%)	19 (43.1%)	43 (48.3%)
Female	21 (46.6%)	25 (56.8%)	46 (51.6%)
Total	45	44	89

**[Table/Fig-1]:** Demographics of study population

Characteristics of the subjects	Cases (n=45)	Controls (n=44)	Student t-test p-value (<0.05)
Age	51.6±9.6	49±12	NS
Sex (male:female)	24:21	19:25	NS
TC	4.46 ± 0.95	4.25 ± 0.72	0.271
TG	1.61 ± 0.92	1.49 ± 0.71	0.479
HDL-c	0.89 ± 0.16	0.99 ± 0.16	0.007**
LDL	2.71 ± 0.91	2.52 ± 0.49	0.240
apo A-I	104.52±16.82	105.54 ± 9.05	0.747
apo B	106.95± 37.81	91.06 ± 22.64	0.020*
apo B/apo A-I	1.00±0.39	0.84 ± 0.18	0.026*
TC/HDL-c	4.81 ± 1.04	4.54 ± 0.83	0.181
TG/HDL-c	1.78±1.01	1.51±0.74	0.180
LDL/HDL-c	3.00 ± 1.01	2.79 ± 0.77	0.282
NonHDL-c	3.81 ± 1.04	3.54 ± 0.83	0.181

**[Table/Fig-2]:** Lipid panel parameters and Apo B/Apo A-I levels of study population

\*Statistically significant \*\*Statistically highly significant. NS = statistically not significant

## DISCUSSION

CVD remains the leading cause of death in the developed and developing countries [16]. Atherosclerosis is one of the major events that leads to development of CVD. Among the various factors for atherosclerosis, major risk factors are high cholesterol level, hypertension, metabolic syndrome and diabetes mellitus [17]. Study has shown the close association of apolipoprotein metabolism with the development of atherosclerosis and apo B/apo A-I ratio to be strong predictor of cardiovascular events than lipid parameters such as TC and LDL-c [18].

In the present study, we tried to figure out the association of apo B/apo A-I ratio with CVD population of Kathmandu valley, Nepal. In addition, we tried to evaluate and compare the predictive value

of apo B/apo A-I ratio and classical lipid profile parameters for development of CVD. Results of the study showed statistically significant difference in apo B/apo A-I ratio among the case and control group. Apo B/apo A-I ratio is significantly higher in patients with known cases of CVD than in participants without CVD. Similarly, HDL-c level is found to be significantly lower in the participants with CVD than in control group, while apo B level showed a reverse association. The lipid profile including TC, TG, LDL-c, non HDL-c and lipid ratios: TG/HDL-c, TC/HDL-c and LDL-c/HDL-c did not show any significant association. In accordance to our finding, high apo B levels with low apo A-I and high apo B/Apo A-I ratio have been found to be strongly associated with CAD risk [18]. Similarly, Meisinger et al., [19] demonstrated a strong prediction of CAD by apo B and the apo B/apo A-I ratio in men and women.

According to the findings of Dawar et al., [20], apo B/apo A-I ratio has been reflected as marker for prediction of risk of myocardial infarction (MI) than traditional lipid ratios. Parallel with our findings, in 2005 Kim et al., in their findings have emphasized stronger risk relationship for apo B/ apo A-I ratio than for other lipid, lipoproteins or lipid ratios [21]. One of the studies carried out in children showed the similar results and apo B/ apo A-I ratio has been shown to be a useful marker to identify those children, particularly boys, with CVD risk factors [22]. In addition, Yusuf et al., have highlighted the significance of apo B/apo A-I ratio and suggested it as a better marker to predict CHD and stroke risk than any of the conventional cholesterol indices [14]. Different studies and published data have suggested apo B/apo A-I as better indicator of risk for vascular disease [23-25]. Therefore, apo B/apo A-I ratio can be the superior alternative for conventional TC/HDL-c ratio for CVD risk assessment [26].

While evaluating the lipid related risk factors for development of CVD, major guidelines have proposed the diagnostic utility of HDL-c, non HDL-c, TG, and lipid ratios such as TC/HDL-c and LDL-c/HDL-c [27]. The Castelli Index (CI) or TC/HDL-c devised by Dr. William Castelli 25 years ago was considered as an excellent predictor of coronary risk [28]. But in recent years different studies have suggested apolipoproteins as more informative lipid risk factors [27] and revealed the greater prognostic value of apo B/apo A-I ratio than that of conventional lipid markers [13].

Among the major drawbacks of lipid profile and lipid ratios we identify some strange observations [Table/Fig-2]. One of the major obstacles while calculating LDL-c using the Friedewald formula [15]  $[TC-(TG/2.2+HDL-c)]$  is that it is not valid when TG is  $>4.2$  mmol/L (450 mg/dL). Similarly, calculation of LDL-c/HDL-c ratio seems strange since HDL-c is included in both numerator and denominator [29]. On the other hand the advantages of using apolipoproteins over classical lipid parameters can be emphasized by the availability of standardised assays that are accurate and automated [30]. Moreover, fasting samples are not mandatory. Besides these, apo B indicates the atherogenic side and apo A-I on the other hand indicates the anti-atherogenic side. Hence, apo B/apo A-I ratio simply reflects the risk of cardiovascular events [13].

Assessment of CVD using better predictors would certainly help those with established disease and those at risk to make proper interventions before being victims of further complications. Our findings showed that apo B/apo A-I ratio is a superior marker for prediction of CVD than classical lipid parameters. Studies published worldwide on favor of apo B/apo A-I ratio have increased the possibility that it may be introduced in routine laboratory investigation for cardiovascular risk assessment. It is also important to evaluate whether it is economically justifiable to introduce it in routine diagnostic tests in a country like Nepal. Further studies are required to investigate if any genetic variants of apo B and apo A-I are associated with increased risk of CVD in Nepalese population. Small population size and lack of follow-up studies are the major limitations of the present study. In addition, we have not

considered other cardiovascular risk factors which might have an equal role for the development of CVD in case group. Hence, taking in consideration of the co-existence of other cardiovascular risk factors, more studies involving large population size is required to establish apo B/apo-I ratio as promising marker for assessment of CVD.

## CONCLUSION

Based upon this study, apo B/apo A-I ratio seems to have better predictive value than that of classical lipid parameters in cardiovascular risk assessment. Measurement of apo B/apo A-I ratio would be more informative to evaluate the cardiovascular events.

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