Coronary heart disease (CHD) is a leading cause of morbidity and mortality globally. There is an increase in the prevalence of CHD in developed as well as developing countries and it has presumed to be the leading cause of death in India by 2020. 1, 2 CHD causes more than 7 million deaths among adult population (21.9 per cent of total deaths, projected to increase to 26.3 per cent by 2030) globally each year, and most of these deaths occurred in developing countries (Figure 1.1). 3

Figure 1.1: Prevalence of CHD (WHO, Preventing chronic diseases, 2005)

According to the estimates of various international bodies like World Bank (WB) and World Health Organization (WHO) the rate of deaths caused by CHD in India will be double in 2015 as compared to the 1985. 4

Epidemiological studies from various parts of India have reported the rising trends and a high burden in the levels of CHD and in its conventional risk factors. 5

The Framingham Heart Study (2010) reported that somewhere between 6.5% and 13% of people living in urban areas and between, 1.6% and 7.4% of rural dwellers in India are living with cardiovascular disease (CVD), and men and women were affected almost equally. 6 Joshi et al. (2009) conducted research in rural Andhra Pradesh and found that 32% of all deaths in that region were occurred due to the cardiovascular diseases, and that 6.6% of the population over 30 years of age had cardiovascular disease. 7
The studies have also showed that India is experiencing an epidemiological health transition characterized by rapid decline in nutritional and parasitic diseases (pre-transitional diseases) and increase in the diseases like CVD.  

Due to this shift India is facing an increased burden of cardiovascular disease. There are various factors responsible for this rising burden of cardiovascular diseases in India. First, due to the development and improved health services, life expectancy in India increased from 40 years in 1951-1961 to almost 62 years in 1991-1996, and with expanding elderly population, the absolute number of CVD cases are also increasing. Second, the life style transition with adoption of unhealthy life style comprising of sedentary habits, lack of physical activity, increased use of transportation and travelling, increasing stress of day to day living, and causing excess smoking and alcohol consumption especially in vast metropolitan cities leads to complications like obesity, hypertension, and abnormal lipid profiles. Third, there is nutritional transition from traditional healthy vegetarian dietary habits to consumption of atherogenic and thrombogenic diet rich in cholesterol and salt is increased. Finally, socio-economic transition associated with urbanization, industrialization and affluence. Additionally, due to the genetic makeup, Asian Indians are ethnically more prone to develop CVD. The various studies performed in migrant South Asians living in United Kingdom, South Africa, Singapore, and North America revealed higher prevalence of cardiovascular diseases in Asian Indian.

All these factors considerably increase the burden of cardiovascular diseases in Indian population. Although, factors like genetic mutation are some of the non-modifiable factors associated but, CVD is mostly related to the modifiable risk factors like unhealthy life style, obesity, diabetes, smoking, and lack of physical activity. Thus, there is an immediate need to modify the life style and nutritional habits to reduce this burden and the early screening and the prediction of cardiovascular diseases is necessary to manage them timely and properly and to decreases the morbidity and mortality related to the CVD.

The risk factors for CHD could be classified as conventional or traditional risk factors, which have been shown to be causally associated with CHD like smoking, diabetes etc. and the emerging or novel risk factors, which have been shown to be associated with CHD, but their usual role in development of CHD is still debated. Schematic representation of risk factors in CHD is presented in Figure 1.2.
Introduction

Figure 1.2: Schematic representation of risk factors in CHD

Such risk factors, which have not been conclusively established to have a cause and effect relationship with CHD, are best described as 'risk markers'. Some risk factors like obesity, which lead to high blood pressure, diabetes, lipid abnormalities are better described as a 'predisposing risk factors'.

Conventionally, the traditional risk markers as LDL-C were widely renowned as an established cardiovascular risk marker. Results of numerous clinical trials demonstrate the capability of LDL-C to independently predict development and progression of coronary heart disease. However, there are subsets of patients who do not have raised lipid profile and normal LDL-C values but still they develop CVD. Extensive researches have been done to determine the underline causes of CVD and risk markers exclusively related to the group of these patients.

Although LDL-C is not typically elevated in the metabolic syndrome, it is highly atherogenic because of the increased presence of small, dense LDL particles. Therefore, measuring only the LDL-C can underestimate the true atherogenic burden. Measurements of apolipoproteins are more accurate, as they quantify the total number of potentially atherogenic particles in plasma. The finding of elevated apolipoprotein B (apoB) in a person with a ‘normal’ LDL-C level likely indicates an increased cardiovascular risk. Apolipoprotein B may also be a more important index than LDL-C in patients with
atherogenic dyslipidemia since it is disproportionately higher in persons with high triglyceride levels.

Apolipoproteins are the central components of lipoprotein particles, and there is accumulating evidence that the measurement of various forms of apolipoproteins like apolipoprotein B can improve the prediction of the risk of cardiovascular disease.\textsuperscript{18} There are strong evidences supporting that the increased serum apolipoprotein B concentration is an important CHD risk marker.\textsuperscript{19} It is a structural protein that constitutes a major component of the very-low-density lipoprotein (VLDL), the intermediate-density lipoprotein (IDL), and the low-density lipoprotein (LDL) (\textbf{Figure 1.3}).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure_1.3.png}
\caption{Constituents of Apolipoprotein B}
\end{figure}

Each of these lipoprotein particles carries one apolipoprotein B molecule; as a result, the total serum apolipoprotein B level corresponds to the total number of VLDL, IDL, and LDL particles. Because VLDL, IDL, and LDL are considered atherogenic, the apolipoprotein B level should reflect the atherogenic potential of these lipoproteins.\textsuperscript{20} Apolipoprotein B is a superior and more potent indicator of CHD risk than LDL and can predict the cardiovascular events more accurately than LDL-C.\textsuperscript{21} It has been reported that apoB hold on to their predictive power even in patients receiving lipid-modifying therapy.\textsuperscript{22} The concentration of plasma apolipoprotein B particles is highly correlated with the level of non-HDL cholesterol (non-HDL-C) and it was reported as a valid surrogate of non-HDL-C and both are equivalently effective to predict the CV events.\textsuperscript{23}
There have been numerous studies present in medical literature with contrasting results for efficacy and use of apo B. Some proved that apo B is better predictor in their studies, while some did not find apo B as a useful and better risk marker than traditional risk factors.

The use of apolipoprotein B as a risk marker in CHD was also advocated by Sniderman et al. They provided three reasons that why apolipoprotein B should be measured for CHD risk assessment? First, it identifies those individuals who have elevated atherogenic particle concentration, independent of their levels of LDL-C levels. Second, measurement apolipoprotein B, allows the clinician to properly classify all of the atherogenic dyslipidemias, including identification of familial combined hyperlipidemia and familial dysbetalipoproteinemia, both of which are associated with a high risk of CVD. Finally, the accuracy of measurement of apolipoprotein B has become ever more reliable relative to traditional lipid measurement. Similarly, in 2008 a Consensus Conference Report from the ADA and the ACC, along with an international expert panel, suggested that measurement of apolipoprotein B be included along with LDL-C and non-HDL-C in a variety of high and highest-risk patients.

In the western countries, high apolipoprotein B and low apolipoprotein A-1(apo A-1) have been shown to be an independent predictor of CHD. As compared with the western world very few studies have been conducted in this regard in Asian countries especially in India. Hence, to fill this gap the present study was undertaken to evaluate apolipoprotein A-1, apolipoprotein B and apo B/apo A-1 as predictors of CHD.

Similarly, non-HDL-C was also proved to be an excellent risk marker of CV events. It has been shown to be a better than any other conventional risk marker in both primary and secondary prevention studies. Elevated non-HDL-Cholesterol signifies increased CVD risk, even if LDL cholesterol levels are at or below the NCEP goal or appear “normal.” In clinical trials, non-HDL cholesterol has been shown to independently predict CHD. Just as LDL is the primary carrier of cholesterol in plasma, two remnant lipoproteins—VLDL and IDL—are the main carriers of triglycerides. These triglyceride-rich lipoproteins (TGRLPs) also carry cholesterol. In the presence of hypertriglyceridemia, TGRLPs may be partly depleted of their triglyceride content and become enriched with cholesterol from LDL. The modified remnant lipoproteins that result are believed to be highly atherogenic because of their small size, high cholesterol
content, and increased residence time in plasma. They are able to deliver more cholesterol to macrophages than LDL particles because they can penetrate the arterial wall with ease, be taken up directly by macrophages, and participate in foam cell formation, thus initiating the lipid-laden plaque. At the same time, LDL exchanges core lipids with VLDL to become triglyceride rich and undergoes lipolysis, resulting in a smaller and denser LDL particle.

These compacted, lipid-depleted LDL particles are more atherogenic because they are more easily oxidized and readily penetrate the artery wall. However, even though the small, dense LDL particles are greater in both number and atherogenicity than normalsized LDL, LDL cholesterol levels appear “normal” rather than “high” on standard measurements because small, dense particles are lipid poor. Therefore, the measurement of LDL cholesterol alone does not provide sufficient measure of atherogenic risk in hypertriglycerideremic patients and a second measure of atherogenic risk is warranted.

Unlike, LDL-C non-HDL-C represents the cholesterol content present in all the atherogenic lipoproteins. In the simple words, it is the sum of Cholesterol accumulated in all lipoproteins, except HDL, such as Chylomicrons, VLDL and their remnants, IDL, LDL and Lp(a) (figure 1.4).

![Cholesterol Content of All Atherogenic Lipoprotein Particles](image)

**Figure 1.4:** Composition of Non-HDL-C
The advocacy for non-HDL-C began following widespread recognition of its superiority over LDL-C as a measurement of CV risk and demonstrated equivalency to apolipoprotein B in various clinical trials. There are several reasons that why the level of non-HDL is superior to that of LDL in CHD risk prediction. First, as discussed above non-HDL cholesterol contains all potential atherogenic lipoproteins, including VLDL, intermediate-density lipoprotein, and LDL, whereas LDL cholesterol does not. So, the use of LDL alone will ignore the contribution of these triglyceride-rich lipoproteins in the development of CHD. Second, in the clinical lipoprotein analysis, the level of LDL cholesterol is usually estimated using Friedewald’s formula, based on the measurements of total and HDL cholesterol and triglycerides, which is used to estimate the value of VLDL cholesterol. However, the estimation of LDL by this formula becomes gradually less accurate as the triglyceride level increases, and the formula is no longer considered accurate enough for use when triglyceride levels reach 400 mg/dl. In contrast, the estimation of non-HDL cholesterol concentrations requires no such assumptions. Finally, the non-HDL level can be calculated in the non fasting state or in the setting of hypertriglyceridemia. Due to these reasons and the benefits of non-HDL-C, the latest recommendations of European and American Cardiological Association give emphasis on the role of non-HDL-C in evaluating the risk of CHD. Moreover, various international societies [(American Diabetes Association (ADA), American College of Cardiology (ACC), and National Lipid Association)] also recommends reporting of non-HDL-C on all lipid profiles, as it incurs no additional expense to the patients.

A little attention is being paid to the use of non-HDL-C and there is much debate over the use of non-HDL-C in routine diagnosis of cardiovascular diseases, as a result we took this as one of the research parameter to evaluate in the present study.

Lipoprotein (a) is another newly identified important risk marker for CHD. Lp(a) is a cholesteryl ester and apolipoprotein B containing particle, which differs from low density lipoprotein (LDL) by the additional presence of a glycoprotein termed apolipoprotein (a), which is homologous to plasminogen. Lp(a) was discovered in human serum in 1963 by Kare Berg during a study of variation in LDL antigenicity. Lp(a) levels have been associated with cardiovascular disease in numerous studies. Recently Lp(a) serum levels were found to be associated with severity of coronary atherosclerosis.
type of LDL which is particularly atherogenic (causes cholesterol deposits in arteries) and also appear to increase the risk of blood clot formation in already narrowed arteries leading to heart attacks or strokes. Lp(a) is dependent on genetic markers and hence levels are often found to be elevated in families with a history of early heart disease. High Lp(a) predicts risk of early atherosclerosis independently of other cardiac risk factors, including LDL. In patients with advanced cardiovascular disease, Lp(a) indicates a coagulant risk of plaque thrombosis. Apo(a) contains domains that are very similar to plasminogen (PLG). Lp(a) accumulates in the vessel wall and inhibits binding of PLG to the cell surface, reducing plasmin generation which increases clotting. This inhibition of PLG by Lp(a) also promotes proliferation of smooth muscle cells. These unique features of Lp(a) suggest Lp(a) causes generation of clots and atherosclerosis.

Lipoprotein ratios were also proved to be the robust risk markers for CHD. The results of large epidemiological and clinical studies showed total cholesterol/HDL, LDL/HDL, TG/HDL and apolipoprotein B/apolipoprotein A-1 to be excellent markers for CHD risk. The routine estimation and evaluation of these ratios can provide information on risk factors difficult to measure by other routine analyses and could be used as a better alternate to traditionally used other routine analyses.

Literature Survey highlights total cholesterol/HDL cholesterol (TC/HDL-C) ratio as a superior risk marker for CHD. The result of these studies proposes that this “cholesterol ratio” is a simple approach for lipid risk assessment. The total cholesterol/HDL cholesterol ratio was proposed as a marker of coronary risk about 25 years ago by Dr. William Castelli and afterwards this ratio is known as Castelli index. An increase in total cholesterol concentration, and specifically LDL cholesterol, is an atherogenic lipid marker, whereas reduced HDL cholesterol concentration is correlated with numerous risk factors, including the components of the metabolic syndrome, and probably involves independent risk. When total cholesterol, HDL cholesterol, and total/HDL cholesterol ratio are compared between an apparently healthy population and myocardial infarction survivors, the total/HDL cholesterol ratio is found to present less superposition of population.

TC/HDL-C ratio is a superior risk predictor for CHD for several reasons. Firstly, the LDL-C level is usually calculated from the Friedewald formula based on the measurement of total cholesterol, HDL cholesterol, and triglycerides. However, for
accurate measurement based on Friedewald formula, a fasting triglyceride level must 200 to 499 mg/dl. In patients of CHD with other alignments like diabetes, there is often elevated triglyceride which results in unreliable LDL-C calculation.\textsuperscript{58} Secondly, measurement of TC/HDL-C is simple and cost effective. Finally, TC/HDL-C estimation does not require fasting samples.\textsuperscript{59} As total cholesterol/HDL ratio is considered a more sensitive and specific index of cardiovascular risk than total cholesterol, the Canadian working group has chosen this lipid ratio as a secondary goal of therapy.\textsuperscript{17}

A more tenable option that has been proven to be an accurate predictor of cardiovascular risk is the LDL-C/HDL-C ratio, which can be obtained from a standard lipid profile and is more accurate than LDL-C or HDL-C alone.\textsuperscript{60} Several large epidemiological and clinical studies have found the LDL-C/HDL-C ratio to be an excellent predictor of CHD risk and an excellent monitor for the effectiveness of lipid-lowering therapies.\textsuperscript{61, 62} The investigators of the Helsinki Heart Study observed that the increase in this ratio predicted a greater cardiovascular risk in a wide range of cholesterol or triglyceride concentrations, the risk is significantly higher when hypertriglyceridemia is present. LDL-C/HDL-C is a better predictor of cardiovascular risk than LDL-C alone.\textsuperscript{60}

The ratio of TG/HDL-C, initially proposed by Gaziano et al.\textsuperscript{63} is an atherogenic index that has proven to be a highly significant independent predictor of myocardial infarction, even stronger than TC/HDL-C and LDL-C/HDL-C. The Copenhagen Male Study showed triglycerides on their own to be another strong risk factor, but it found that stratifying triglyceride levels by HDL-c levels led to more accurate detection of increased risk of coronary disease.\textsuperscript{64}

The atherogenic link between high triglycerides and HDL-C is due to the higher plasma concentration of triglyceride-rich, very low-density lipoprotein that generates small, dense LDL during lipid exchange and lipolysis. These LDL particles accumulate in the circulation and form small, dense HDL particles, which undergo accelerated catabolism, thus closing the atherogenic circle.\textsuperscript{65, 66}

Recently, this ratio has been suggested to an easy, non-invasive means of predicting the presence and extent of coronary atherosclerosis \textsuperscript{67} and also proposed to be a better atherogenic marker than conventional risk markers \textsuperscript{68}. 

\textsuperscript{58} Friedewald, \textsuperscript{59} Friedewald, \textsuperscript{17} Friedewald, \textsuperscript{60} Friedewald, \textsuperscript{61} Friedewald, \textsuperscript{62} Friedewald, \textsuperscript{63} Friedewald, \textsuperscript{64} Friedewald, \textsuperscript{65} Friedewald, \textsuperscript{66} Friedewald, \textsuperscript{67} Friedewald, \textsuperscript{68} Friedewald.
The apolipoprotein B/apolipoprotein A-1 ratio was suggested as a better risk predictor for CHD, than any of the conventional lipid ratios. The result of various studies showed that the value of apoB/apoA-1 ratio was significantly related to the risk of CVD and for this reason the apoB/apoA-1 ratio was suggested as an alternative to other lipid and lipoproteins ratios for risk assessment in patients with CHD.\textsuperscript{69,70} The early efforts to standardize and improve apolipoprotein B, apolipoprotein A-1 and their ratio measurement were initiated in 1981 through a collaborative effort between the CDC and the International Union of Immunological Societies.\textsuperscript{71} Further work to standardize apolipoprotein B, apolipoprotein A-1 and their ratio measurements were conducted by the Northwest Lipid Research Laboratories (NWLRL) at the University of Washington, Seattle, WA, under the patronage of the IFCC.\textsuperscript{72} Apolipoprotein B/apolipoprotein A-1 reflect the relationship between proatherogenic apo B-containing lipoproteins and anti-atherogenic HDL fractions. This also believed to be a more reliable parameter for measuring HDL than cholesterol content since it is not subject to variation. The advantage of calculating this index is that the concentration of apolipoproteins does not change after the meal and do not change in different times of day.\textsuperscript{54} Furthermore, this ratio reflects the balance between two completely opposite processes: transport of cholesterol to peripheral tissues, with its subsequent arterial internalization, and reverse transport to the liver.\textsuperscript{73} The greater the apolipoprotein B/apolipoprotein A-1 ratio, the larger will be the amount of cholesterol from atherogenic lipoproteins circulating through the plasma compartment and likely to induce endothelial Vascular Health and Risk Management dysfunction and trigger the atherogenic process.\textsuperscript{74}

The non-HDL/HDL-C is another lipid ratio, which has a good potential to predict the CV events. There are very few studies describing the utility and efficacy of this ratio in CHD. Eliasson B et al in their study concluded that lower levels of non-HDL-C/HDL-C are a better risk marker for CHD than LDL-cholesterol.\textsuperscript{75} Similarly, Kim SW et al in their study showed that the non-HDL-C/HDL-C ratio is a better marker for identifying insulin resistance and metabolic syndrome.\textsuperscript{76} This is perhaps the first Indian study leading to diagnostic correlation of this ratio in CHD.
The present study was conducted to evaluate the role of these newly identified risk markers associated with CHD in patients aged 20 years to 60 years. The work was mainly focused on emerging or novel risk markers like, NON-HDL-C, apoliporotein B, apoliporotein A-1 and Lp(a) and the ratio of non-HDL-C/HDL-C in CHD patients. The evaluation of coronary risk markers associated with atherogenesis is continuing. Some patients without any known risk factor also develop CHD. Therefore, the role of these newly identified risk markers in such group of patient was evaluated in the present study.