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COMPARATIVE STUDY OF SERUM APOLIPOPROTEIN A_1 AND hsCRP IN MYOCARDIAL INFARCTION

Biochemistry

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ABSTRACT

Background: Apolipoprotein A1, is a major apoprotein of HDL or so called "Good cholesterol".HDL itself posseses reverse cholesterol transport producing an antiatherogenic effect in body.hsCRP or high sensitive C reactive protein is an acute phase protein which has atherogenic property. The aim of the study is to evaluate concentrations of Apo A1 and hsCRP among the ischemic heart disease patients and healthy controls and to provide inter-relationships between Apo A1, and hsCRP among the patients. **Methods:** A case control study was performed in the department of biochemistry in collaboration with department of medicine, Shija Hospital and Research Institute, Imphal,Manipur.60 ischemic heart disease patients and 60 healthy controls were taken for the study population. **Results:** the results of this tudy shows that the serum levels of Apo A1 and hsCRP are altered significantly between cases and controls and no significant correlation has been found between these two cardiac biomarker in this study. **Conclusions:** The present data represents the measurement of hsCRP as an important screening tool for the risk measurement of ischemic heart disease.

KEYWORDS

Apolipoprotein A1, hsCRP, ELISA., Myocardial infarction.

INTRODUCTION

Acute Myocardial Infarction (MI) is one of the major causes of morbidity and mortality in the world and atherosclerosis is the major cause of ischemic heart disease.[1] Ischemic Heart Disease (IHD) is a condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium; it typically occurs when there is an imbalance between myocardial oxygen supply and demand.

The most common cause of myocardial ischemia is atherosclerotic disease of an epicardial coronary artery (or arteries) sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery. Hyperlipidemias and hyperlipoproteinemias are the most important risk factors for atherosclerosis. IHD causes more deaths and disability and incurs greater economic costs than any other illness in the developed world.[2]

There are few studies on serum concentrations of apolipoprotein A_1 and hsCRP and their interrelationships in IHD. Some studies showed that these individual parameters are independent and continuous risk variables for IHD and some showed that these are not independent risk factors. Hence, the present study is carried out to evaluate the relationship between apolipoprotein A_1 and hsCRP in patients with IHD.

AIMS AND OBJECTIVES

A) To evaluate:
i) Concentrations of Apolipoprotein A_1 (Apo A_1)
ii) Concentrations of high sensitive C Reactive Protein(hsCRP) in Ischemic Heart Disease patients and healthy controls .

B) To establish:
i) Inter-relationships between Apolipoprotein A_1, high sensitive C Reactive Protein among the patients.

MATERIALS AND METHODS

The study has been conducted after the approval from the institutional ethical committee. The informed consent of the patients or their relatives has been taken prior to the inclusion.

Study design: Case control study

Study Settings:
The study has been conducted in the Department of Biochemistry in collaboration with the Department of Medicine, Shija Hospital and Research Institute, Imphal,Manipur.

Study population:
Consecutive patients with IHD attending SHRI, Imphal.

Subjects:
A total number of 120 subjects participated in the study, of which 60 were cases and 60 were controls.

Inclusion Criteria
- All patients with already diagnosed ischemic heart disease who attended Cardiac clinic/Medicine OPD or admitted in the Medicine ward or ICCU.
- All patients above 18 yrs of age, irrespective of sex, religion and socio economic status.

Exclusion criteria
Ischemic heart disease patients with
- Hepatic and renal disease
- Sepsis
- Malignancy
- Diabetes mellitus
- Anemia
- Hypolipidemic drugs

Collection Of Blood Samples:
About 6 ml of venous blood was drawn under aseptic conditions in a sterile vacationer from selected subjects after a period of overnight fasting of 12 hours. Serum was immediately separated by centrifugation and used for analysis of Apolipoprotein A_1, and hsCRP.

Human Subject Protection:
There was no physical risk to the participants in the study. The participants were informed that their participation was voluntary and that they were free to withdraw at any time. The study was approved by the Research Ethics Board of RIMS, Imphal.

Statistical Analysis:
Statistical analysis was done using SPSS version-20 software. Results were reported as mean $\pm$ SD (standard deviation) for quantitative variables and number of cases along with percentages for the categorical/quantitative variables.

Chi-square test, independent sample T test were applied whenever necessary. All comparisons were two-sided and the P- values of < 0.05 and < 0.01 were used as the cut-off values for significance and highly significance respectively.

Ethical Issues:
- Approval was sought from Research Ethics Board, SHRI, Imphal.
- Consent taken before taking blood samples.
- Confidentiality maintained.

Materials
Serum Apolipoprotein A_1 and hscrp were measured by using KinesisDX's Enzyme Linked Immunosorbent assay ELISA^{PRO}KIT and DBC ELISA pro kit respectively,,manufactured by KinesisDX Los Angeles, CA 90007, USA, and marketed by KinesisDX India Private Limited, as described by Nakajima K et al.[3]

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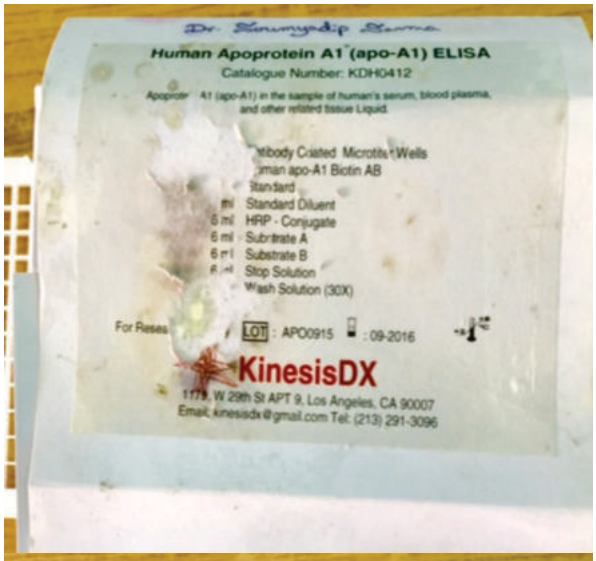
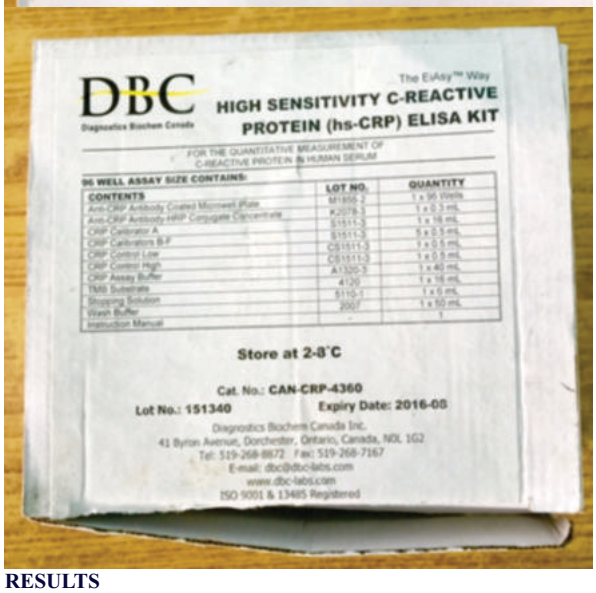




RESULTS

Table 1. Variation Of The Concentration Of Apolipoprotein A1 And hsCRP Among Different Age Groups In Case And Control:

	31-40 years	41-50 years	51-60 years	>60 years	ANOVA significance
Apo A1 (µg/ml)	1371.42±447.95 µg/ml	1211.76±706.11 µg/ml	1199.03±718.82 µg/ml	1282.43±1105.05 µg/ml	f=0.19 p=0.90
hsCRP (µg/ml)	1.55±0.97 µg/ml	2.13±0.95 µg/ml	2.44±1.76 µg/ml	2.33±1.56 µg/ml	f=1.30 p=0.27

Table 2. Variation Of The Concentration Of Apolipoprotein A1 And hsCRP Among Both Sex Groups In Case And Comtrol:

	Male(32)	*Female(28)*	*Significance t test*
Apo A1 (µg/ml)	1265.75±676.63 µg/ml	1217.02±1024.39 µg/ml	t=0.314 p=0.754
hsCRP (µg/ml)	2.18±1.55µg/ml	2.37±1.52µg/ml	t=-0.678 p=-0.499

Table 3.Distribution Of The Respondents By Serum Concentrations Of APO A1 and hsCRP

Variable	Mean±SD
Ischemic heart disease	
Apo A1	748.3±821.7µg/ml
hsCRP	6.5±1.6 µg/ml
Healthy controls	
Apo A1	1745.0±444.5 µg/ml
hsCRP	1.2±0.5 ng/ml

Table 1 shows variation of the concentration of apolipoprotein A1 ans hsCRP among different age groups where Apo A1 concentration gradually decreases.The concentration of hsCRP is gradually increases from lower age group to group of 51-60 years and thereafter it is found to be decreased in the age group of >60 years.Comparison between different aage groups among cases and controls by ANOVA test was statistically insignificant (p>0.05).

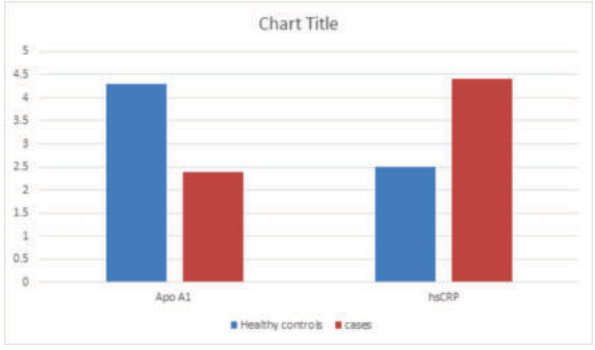
Table 4. Correlation between Apo A1 and hsCRP

Variable	Mean ± SD	Unpaired t-test
ApoA1 (µg/ml)		
case	748,3±821.7µg/ml	t=8.2
control	1745±444.5µg/ml	p-<0.001
hsCRP (µg/ml)		
case	3.2±1.6µg/ml	t=8.89
control	1.2±0.5 µg/ml	p-<0.001

Table 2 shows varation of the concentration of apolipoprotein A1 ans hsCRP among both male and female groups in study group,where apoA1 has been found significantly higher among males(32),and hsCRP has been found higher among females(28) which has been found insignificant (p>0.05).

Table 3 depicts that mran with standard deviation of apo A1 and hsCRP were 748.3±821.7 µG/ML AND 6.5±1.6 µG/ML respectively for ischemic heart disease and for healthy controls 1745.0±444.5 µg/ml and 1.2 ±0.5 µg/ml.

Table 4 significantly shows that on comparing various level of Apo A1 and hsCRP of cases with the controls,for Apo A1 cases had lower level than controls(748.3±821.7 vs 1745±444.5) but for hsCRP cases had higher values than controls.All the findings were statistically significant (p<0.05).



DISCUSSION

The data for this study was collected from Baroda Heart Institute & Research Centre (BHIRC). The total 208 patients were considered for study of CAD.The majority of the patients were from age group of 60-69 years i.e. 67 (32.21%) and only 10 patients (4.32%) were in the age group of ≥80 years. Amongst all patients, 93.27% were lacking exercise, 39.42% were hypertensive, 28.85% were obese, 27.88% diabetic, 10.58% had family history of CAD and 5.28% were smokers. It is essential to identify CAD risk factors amongst the Indians to tackle the problem. So this study was of great help in this direction.

Similar study by Jackson CF et al[4] who has found prevalence of IHD in the same age group in a case control study. The aging of the population worldwide will result in increasing numbers of elderly patients, among whom heart disease is the leading cause of death. Changes in cardiovascular physiology with normal aging and prevalent co-morbidities result in differences in the effects of common cardiac problems as well as the response to their treatments. Patient-centered goals of care such as maintenance of independence and reduction of symptoms may be preferred over increased longevity. New less-invasive treatments are likely to improve outcomes in elderly patients who previously have been considered at prohibitive risk for traditional procedures. Clinical trials enrolling elderly patients are limited and recommendations for management from younger patients frequently lack evidence-based support in patients aged >75 years.

The study by Talmud PJ et al[5] who compared the pairwise combinations of total cholesterol, TGL, apolipoprotein B, HDL-C, LDL-C and apolipoprotein A_1,apolipoprotein B_{100} and hsCRP on CHD risk prediction in middle -aged men and younger age groups and concluded aforementioned markers significantly altered with increment of age so that the combined evaluation of apolipoprotein B

with TGs provides useful diagnostic criteria for CHD.

Ridker PM et al[6] reported that C reactive protein level is a stronger predictor of cardiovascular events than the LDL cholesterol level among middle age group than yonger counterpart in a comparative study. Ariyo AA et al[7] observed in older U.S. adults that all Apolipoproteins play an important part in atherothrombogenesis and predicting increased risk of vascular disease in older men.

Kamstrup PR et al[8] reported that extreme apolipoprotein levels predict a 3 to 4 fold increase in risk of MI in the general population and absolute 10-year risks of 20% and 35% in high risk women and men and male have a higher chance of having IHD as compared to female.

Nissen SE et al[9] showed that although low levels of high-density lipoprotein cholesterol (HDL-C) increase risk for coronary disease. Apolipoprotein A_1 milano is a variant of apolipoprotein A_1 identified in individuals in rural Italy who exhibit very low levels of HDL. Infusion of recombinant apolipoprotein A_1 milano-phospholipid complexes produces rapid regression of atherosclerosis in patient models. A recombinant apolipoprotein A_1 milano/phospholipid complex (ETC-216) administered intravenously for 5 doses at weekly intervals produced significant regression of coronary atherosclerosis as measured by IVUS. Although promising, these results require confirmation in larger clinical trials with morbidity and mortality end points. In a small village in northern Italy called Limone sul Garda live approximately 40 carriers with a naturally occurring variant of apolipoprotein A_1 known as apolipoprotein A_1 milano. Individuals with apolipoprotein A_1 milano are characterized by very low levels of high-density lipoprotein cholesterol (HDL-C) (10-30 mg/dl [0.25-0.78 mmol/l]), apparent longevity, and much less atherosclerosis than expected for their HDL-C levels. The apolipoprotein A_1 milano protein differs from native apolipoprotein A_1 in that cysteine is substituted at position 173 for arginine allowing disulfide-linked dimer formation. Recombinant apolipoprotein A_1 milano has been formulated in a complex with a naturally occurring phospholipid to mimic the properties of nascent HDL (ETC-216, Esperion Therapeutics, Ann Arbor, Mich).

Joshi R et al[10] found mortality rates of nearly 31% for ischemic heart disease and stroke combined in rural Andhra Pradesh; however, according to the Global Burden of Disease estimates, ischemic heart disease and stroke deaths are estimated to be approximately 20% and 10%, respectively, for the South Asian Region, which includes India. Other studies in India around the same time as the Global Burden of Disease study suggest that the prevalence of and death rates due to CVD are as much as 2 to 3 times higher in urban areas than in rural areas. If this is true, we may be grossly underestimating the burden of CVD in South Asian countries today. In these aforementioned studies including ours, non healthy lifestyle with smoking and alcohol can be causative factors.

This finding is supported by Finegold JA et al[11] who analysed IHD deaths world-wide between 1995 and 2009 and used the UN population database to calculate age-specific and directly and indirectly age-standardised IHD mortality rates by country and region.IHD is the single largest cause of death worldwide, causing 7,249,000 deaths in 2008, 12.7% of total global mortality. There is more than 20-fold variation in IHD mortality rates between countries. Highest IHD mortality rates are in Eastern Europe and Central Asian countries within middle and lower economic status groups; lowest rates in high income countries. For the working-age population, IHD mortality rates are markedly higher in low-and-middle income countries than in high income countries.Over the last 25 years, age-standardised IHD mortality has fallen by more than half in high income countries, but the trend is flat or increasing in some low-and-middle income countries. Low-and-middle income countries now account for more than 80% of global IHD deaths.The global burden of IHD deaths has shifted to low-and-middle income countries as lifestyles approach those of high income countries. In high income countries, population ageing maintains IHD as the leading cause of death. Nevertheless, the progressive decline in age-standardised IHD mortality in high income countries showed that increasing IHD mortality is not inevitable. The 20-fold mortality difference between countries, and the temporal trends, may hold vital clues for handling IHD epidemic which is migratory, and still growing.

Since economic status mostly related to day to day activity also, Leddy J et al[12] showed dietary fat may be associated with coronary heart disease (CHD) among groups from higher economic status. Studies suggested that restricting fat intake may compromise endurance performance and that increasing fat intake may improve endurance performance. They studied the effects of varying dietary fat intake on CHD risk factors in workers. Twelve male and 13 female workers from lower economic status increased fat from 16% to 30% of daily calories (4 wk each). Of this group, six males and six females increased fat to 42% of daily calories (4 wk). Physiological and lipoprotein risk factors were measured after each diet. Results were analyzed by repeated measures ANOVA. Increasing dietary fat from 16% to 42% of daily calories did not change adiposity, weight, heart rate, blood pressure, serum triglycerides, total cholesterol, LDL cholesterol. apolipoprotein B, or the apolipoprotein A_1/ apolipoprotein B ratio. Compared with those eating higher fat, subjects eating 16% fat had lower HDL cholesterol (50 +/- 3 vs 62 +/- 3 mg.dl-1, $P < 0.0001$) and apolipoprotein A_1 (111 +/- 6 v. 134 +/- 6 mg/dl, $P < 0.0005$) and a higher TC/HDL-C ratio (4.05 +/- 0.27 vs 3.42 +/- 0.24, $P < 0.0005$). Runners who increased fat intake to 42% further raised HDL cholesterol (64 +/- 6 to 69 +/- 5 mg.dl-1, $P < 0.04$) without adversely affecting other lipoproteins. we see that the mean serum concentrations of hsCRP in controls and in patients with ischemic heart disease was in the range of 3.2 ± 1.6 and 1.2 ± 0.5 µg/mL, respectively. The concentration of hsCRP was increased in patients with ischemic heart disease when compared to healthy normal individuals. This study showed a positive association between ischemic heart disease and hsCRP level and the results were in agreement with those of Chowta MN et al[96] Main objective was to correlate the highly sensitive CRP (hsCRP) level to individual components of metabolic syndrome and coronary vascular disease. Forty patients who were diagnosed clinically with metabolic syndrome were included in the study. Detailed history with regard to diabetes mellitus, hypertension and other CVD was collected from each patient. All the patients underwent complete physical examination, including ECG. Height, weight, fasting blood glucose and lipid levels were measured in all the patients. CVD was assessed with the following: new-onset angina, fatal and non-fatal myocardial infarction or stroke, transient ischemic attack, heart failure or intermittent claudication. In the findings**,** the mean hsCRP level was higher in patients with CVD compared with those without CVD. The CRP level correlation with CVD showed a statistically significant correlation. hsCRP level was very high in eight hypertensive patients, whereas it was very high in five normotensives. But, statistical analysis has not shown any significant correlation between hypertension and hsCRP level. Similarly, although a higher hsCRP level was seen in diabeteics, statistical analysis failed to show a significant correlation between diabetes and the hsCRP level. Analyses of hsCRP correlation with body mass index, fasting glucose, cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein did not show a significant correlation with the hsCRP level. It could be concluded that increased hsCRP levels are associated with an increase in the incidence of CVDs. Higher values of hsCRP were observed in patients with hypertension and diabetes. No correlation was seen between hsCRP and components of the metabolic syndrome.

Another study by Sabatine MS et al[13] supports our result where they measured hsCRP in 3771 patients with stable coronary artery disease from the Prevention of Events With Angiotensin-Converting Enzyme Inhibition (PEACE) trial, a randomized placebo-controlled trial of the angiotensin-converting enzyme inhibitor trandolapril. Patients were followed up for a median of 4.8 years for cardiovascular death, myocardial infarction, or stroke, as well as new heart failure and diabetes. After adjustment for baseline characteristics and treatments, higher hs-CRP levels, even >1 mg/L, were associated with a significantly greater risk of cardiovascular death, myocardial infarction, or stroke (hs-CRP 1 to 3 mg/L: adjusted hazard ratio, 1.39; 95% CI, 1.06 to 1.81; P=0.016; hs-CRP >3 mg/L: adjusted hazard ratio, 1.52; 95% CI, 1.15 to 2.02; P=0.003). Similarly, elevated hs-CRP levels were an independent predictor of new heart failure (adjusted P<0.001 for trend) and new diabetes (adjusted P<0.001 for trend). There were no significant interactions between hs-CRP levels and the effects of trandolapril on any of the above outcomes.

Third study is by Ghodke SS et al[14] Who aimed to evaluate the relationship between pre procedural high sensitive C- reactive protein and angiographic signs in patients presenting with acute coronary artery disease. The diagnosis was based on the ECG and enzyme profile. Increased total LDH level was taken as criteria for selection of patients. Total 100 cases were studied with coronary artery disease in

the age group 40 to 70 years. They were followed for 7 days or until discharge. The high sensitive Creactive protein levels were estimated in these 100 patients and the results were compared with 100 age and sex matched healthy controls in the same age group. The mean value of high sensitive C- reactive protein was 4.63±1.43 was significantly higher ($p<0.001$) when compared to healthy controls.

Clearfield MB[15] conducted a study with the aid of The Centers for Disease Control and Prevention and the American Heart Association who therefore proposed joint guidelines for the use of hs-CRP in determining cardiovascular disease risk. The author reviews numerous studies examining the prognostic value of hs-CRP and outlines ongoing efforts to assess the effect of statin therapy in healthy individuals with low levels of low-density lipoprotein cholesterol and high levels of hs-CRP.

Another study done by Ibrahim MM et al[16] conducted in Egyptian patients with IHD.Plasma lipid profile may differ according to ethnic origin and geographic area.The main objective was to identify plasma lipid abnormalities in Egyptians with CAD and define the role of age, type of CAD, and the presence of hypertension (HT) on lipid profile.A Retrospective consecutive sampling of lipid profile of 1000 patients with CAD. Results were compared to a control group of 1920 non-coronary individuals. Patients' age range was 19–90 years. HT was present in 56.7% of patients. The commonest isolated lipid abnormality was a reduced Apolipoprotein A_1 in men and increased plasma triglycerides (TG) in women. Patients with myocardial infarction (MI) had a lower Apolipoprotein A_1 than those with angina pectoris (AP). Abnormalities were more severe and more prevalent in the young age group. No significant difference in lipid profile was present between normotensive (NT) and hypertensive (HT) CAD patients.So by this study it could be concluded that Dyslipidemia is common among Egyptians with CAD. Lipid profile was influenced by age, gender, type of CAD, but not by the presence of HT. The high prevalence rate of risk factors particularly among young Egyptians is remarkable and can explain the epidemic of CAD among Egyptians.

CONCLUSION

In view of the increasing numbers of cardiovascular diseases in this part of the country , the present study was undertaken to find out any correlation between serum Apolipoprotein A_1 and hsCRP and to compare their values with control and patients with ischemic heart disease.

This is the first study of serum Apolipoprotein A_1 and hsCRP in Indian Manipuri population with ischemic heart disease, and this study agrees with previous findings of other studies that serum Apolipoprotein A_1 and hsCRP levels are significantly altered between IHD patients and controls. However, no significant correlation has been found between these three cardiac biomarker in the present study.

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