Section A-Research paper
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HIGH SENSITIVE C-REACTIVE PROTEIN (hsCRP) A SUPERIOR BIO MARKER FOR ASSESING THE RISK OF CORONARY ARTERY DISEASE AMONG T2DM PATIENTS

***M. Chitra Devi[1], G.Sridevi[2], Ponnulakshmi Rajagopal[3], V.Rajalaxmi[4]**

[1]Tutor, Department of Physiology, Government Medical College Krishnagiri, Krishnagiri, Tamil Nadu, India. E-Mail ID: chitrakmc@gmail.com

[2]Professor and Head, Department of Physiology, SRM Dental College, Ramapuram, Chennai. Email: sridevig1@srmist.edu.in

[3]Scientist Grade-III Department of Central Research Laboratory, MeenakshiAmmal Dental College and Hospitals, Meenakhsi Academy of Higher Education and Research (MAHER), Chennai

[4]Professor, Faculty of Physiotherapy, Dr. MGR. Educational and Research Institute, Chennai, Tamil Nadu, India

*Corresponding Author: Dr.M.Chitra Devi,
Address: Department of Physiology,
Govt Medical College Krishnagiri, Krishnagiri, Tamil Nadu, India
E-Mail ID: chitrakmc@gmail.com

ABSTRACT

AIM

To investigate the importance of High sensitivity C reactive protein in predicting the risk of Coronary Artery Disease in Type II Diabetes Mellitus patients. **MATERIALS AND METHODS:** This research was carried out at Supa Hospital, Cardiac Care Centre, Dharmapuri, and Meenakshi Medical College Hospital & Research Institute, Kanchipuram, Tamil Nadu. A total of 60 individuals consist of 20 apparently healthy individuals, 20 Type II Diabetes Mellitus patients (T2DM) and 20 T2DM patients diagnosed with Coronary Artery Disease (CAD) were selected for this research. Anthropometric measurements as well as resting blood pressure were taken along with detailed clinical history. All fasting lipid profiles were analysed using Chemwell 2910 Automated EIA and chemistry analyser. ELISA was used to measure high sensitive C reactive protein (hs-CRP) with a coefficient of variation under 5%. **RESULTS:** This research provides a comprehensive review of current knowledge concerning risk of developing CAD, in evaluating the hs-CRP levels and concludes with a risk assessment of coronary artery disease based on evidence for hs-CRP. To conclude, When compared to normal and T2DM subjects, hs-CRP levels in T2DM patients with CAD were shown to be considerably higher. (Key words: Coronary Artery Disease, T2DM, hs-CRP, Atherosclerosis, Lipid Profile, Blood Pressure).

KEY WORDS: hsCRP; T2DM; Coronary Artery Disease (CAD).

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INTRODUCTION AND OBJECTIVE:

Acute coronary syndrome, stroke, and peripheral arterial disease are signs of atherosclerosis, a chronic inflammatory disease of the artery wall. [1] It is caused by white blood cells called macrophages, which are first dispatched by the body's immune system to remove LDL cholesterol pockets, and fat that has accumulated in the arteries. These white blood cells release a substance called netrin-1 when they adhere to an artery, preventing macrophages from leaving the arteries as they normally would. By preventing macrophages from leaving plaques, the neuroimmune guidance cue netrin-1 encourages atherosclerosis[2]. This plaque obstructs blood flow, which raises the risk of coronary artery disease. Hs-CRP may play a complicated, proatherogenic role in plaque deposition by acting on a wide range of atherosclerotic cell types[3]. hsCRP may promote monocyte transmigration into the vessel wall, a crucial early stage in the atherosclerotic process, and monocyte adherence[4].

Coronary Artery Disease has overtaken all other causes of morbidity and mortality due to its increasing prevalence. Sedentary behaviour, insulin resistance, hyperglycemia, diabetic dyslipidemia, hypertension, hyperinsulinemia, and systemic inflammation are some of the risk factors for coronary artery disease. Inflammation raises blood levels of high sensitive C-Reactive Protein. The 'golden marker for inflammation' has been identified as hs-CRP, which is produced by the liver. Elevated levels of hs-CRP suggest chronic low-grade inflammation, which is thought to have a role in the aetiology and presentation of coronary artery disease[5,6].

Minor CRP elevation [high-sensitivity CRP (hs-CRP)] has been demonstrated to be associated with future substantial cardiovascular risk based on numerous epidemiological and intervention studies (hs-CRP: 1 mg/l = low risk; 1-3 mg/l = intermediate risk; 3-10 mg/l = high risk; >10 mg/l = unspecific elevation). According to the American Heart Association, patients with a high or intermediate risk of coronary heart disease may benefit from having their hs-CRP levels measured in order to determine their personal risk. Patients with all stages of the metabolic syndrome are more likely to develop type 2 diabetes when their hs-CRP levels are elevated[7].

A new strategy for identifying those who are at high risk of developing CAD may be made possible by hs-CRP levels[8]. Few prospective studies show that hs-CRP is a potent independent predictor of future myocardial infarction in apparently healthy men and women, and that adding hs-CRP testing to routine lipid screening may enhance CAD patients' ability to anticipate their overall risk.

Increasing evidence suggest that local and general inflammation has got an significant role in formation, progression and rupture of atherosclerotic lesion[9,10]. The precise processes underlying this inflammatory process are yet unknown, despite the fact that inflammation is implicated in the initiation, progression, and complications of the atherosclerotic process. There are numerous CAD risk prediction studies/tools like Framingham, PROCAM, SCORE, Reynolds, VILCAD, QRISK[11]. Though the Framington Heart Study based prognostic algorithms significantly improved coronary artery disease risk assessment, one third of patients with coronary events have none or one CAD risk aspect, Moreover, 40% of patients who died due to CAD had blood cholesterol concentration lower than average[12]. Hence, it becomes relevant to explore for new and more accurate CAD risk factors in T2DM patients.

Though more and more evidence-based guidelines recommend measuring hs-CRP concentration to predict CAD risk, the data about its benefit remain controversial. In the absence of other biochemical markers, the present study aimed to evaluate the predictive and diagnostic role of hs-CRP in Coronary Artery Disease among T2DM patients.

MATERIALS AND METHODS

Design of the study:

A total of 60 subjects of age group between 40 and 60 were recruited for the study. Based on the presence or absence of CAD, T2DM patients were categorised as follows. Group I- 20 healthy individuals as Control subjects; Group II- 20 T2DM subjects; Group III- 20 T2DM patients were diagnosed with CAD. With the institutional ethical committee's consent, the study was started. After thoroughly outlining the steps and obtaining everyone's informed consent, the study was conducted.

Selection Criteria

Group-I

The following factors were used to choose the control subjects. They had no history of T2DM or CAD, did not smoke or drink, had no endocrine disorders, hyperlipidaemia, or hypertension, and were not taking any medications.

Group-II

Type 2 DM subjects were diagnosed according to world health organization (2015) criteria. when the two-hour post-meal glucose level was greater than 200 mg/dl or the fasting blood glucose level was greater than 126 mg/dl. These subjects had no history of CAD and had normal ECG during exercise, and were on oral hypoglycemic drugs / insulin for more than 6 months.

Group-III

Those who had undergone coronary angiography for suspected ischemic heart disease and met the following criteria were considered to have CAD: 75% or more organic stenosis of at least one major coronary artery; (i) A favourable ECG exercise stress test result. ECG alterations that might indicate CAD. Patients with acute coronary syndrome (iii). (iv) Previous cardiac procedures, such as coronary artery bypass graft (CABG), percutaneous coronary intervention (PCI), and percutaneous transluminal coronary angioplasty.

Chronic alcoholics, patients with severe obesity, and people with BMIs greater than 35 kg/m2 were also excluded. patients with inflammatory conditions, hypothyroidism, endocrinopathies, dilated cardiomyopathy, valvular disease, pre-existing hepatic disease, and cerebrovascular conditions.

After the selection criteria, for all the 3 group subjects, a detailed history was obtained. The protocols involved,

(a) Measurement of Anthropometric Indices

The patients' height and weight were recorded while they were standing and dressed comfortably. To the nearest 0.5 kg, body weight was measured in kilogrammes. The closest 0.5 cm in centimetres were used to measure height. Using Quetelet's Index, the Body Mass

Index (BMI) was calculated as the body weight in kilogrammes divided by the square of the height in metres^2.

(b) Fasting Lipid profile

ChemWell® 2910 Automated EIA and Chemistry Analyzer was used to measure total cholesterol, triglycerides, and HDL cholesterol. For participants with a serum TG content of less than 400 mg/ml, low density lipoprotein cholesterol was indirectly determined using the Friedewald formula (LDL cholesterol=Total cholesterol-HDL cholesterol+1/5 Triglycerides).

(c) Serum hs-CRP

Serum hs-CRP concentration was measured by Enzyme Linked Immunosorbent Assay (ELISA) using commercially available human hs-CRP ELISA Kit (Cat. No. E-80HS-CRP – Lot# 14C1Q1). The assay was based on the principle of the double antibody sandwich ELISA.

Statistical Analysis

After analysis, all data were reported as Mean±SE. SPSS for Windows version 21 was used to perform the statistics. One-way analysis of variance (ANOVA) was used to assess significant differences between groups, and the Duncan's test was used for post hoc comparisons between each group. In order to assess the association between serum hs-CRP and all other research variables, Pearson correlation coefficients were computed. A p value of less than 0.05 was deemed statistically significant.

RESULTS:

Table 1 presents the anthropometric and biochemical traits of the patients and control group based on statistical analysis. This study results are confirming that, among all the study groups, anthropometric characters like age, BMI and biochemical characteristics like SBP, DPP, TG, TC, TC/HDL-C, LDL-C positively correlates with the levels of hs-CRP.

Wherein, HDL-C inversely correlated with the levels of hs-CRP. HDL-C level in control subjects was 48.35^{a}±1.21, HDL-C in T2DM patients group was 34.55^{b}±1.47 and HDL-C in T2DM patients with CAD was 33.00^{c}±0.59. HDL-C levels were comparatively lower in T2DM patients diagnosed with CAD. This research work outlines that, reduced levels of HDL-C and increased levels of hs-CRP may reflect inflammatory effects, which may lead to development of coronary artery diseases.

Table 1. Anthropometric and biochemical characteristics of patients and control subjects.

Parameter	Group-I (Control)	Group-II (T2DM)	Group-III (T2DM+ CAD)	P value
hs-CRP, mg/l	0.75^{a}±0.43	1.48^{b}±0.10	3.33^{c}±0.10	0.000
Age, years	48.45^{a} ± 1.57	50.25^{b} ±5.03	51.75^{c} ± 1.40	0.246
BMI, kg/m2	22.62^{a}±0.35	24.89^{b}±0.59	29.08^{c}±0.43	0.000
SBP, mmHg	115.05^{a}±0.97	122.40^{b}±0.54	135.85^{c}±1.14	0.000
DBP, mmHg	72.35^{a}±0.75	73.25^{b}±0.70	80.80^{c}±1.07	0.000
TG, mg/dl	131.10^{a}±3.08	202.00^{b}±4.01	207.85^{c}±5.81	0.000
TC, mg/dl	179.60^{a}±1.96	194.15^{b}±3.32	200.50^{c}±2.82	0.000
HDL ,mg/dl	48.35^{a}±1.21	34.55^{b}±1.47	33.00^{c}±0.59	0.000

TC /HDL-C	3.76^{a}±0.11	5.77^{b}±0.21	6.12^{c}±0.15	0.000
LDL-C , mg/dl	105.65^{a}±2.55	124.35^{b}±2.84	143.9^{c}±5.70	0.000

^{abc} Means with successively different superscripts differ significantly**P 0.01*. ANOVA analysis was used for the statistical analysis (post hoc test: Duncans).

Correlation of levels of hs-CRP

The findings of this investigation showed that T2DM patients and T2DM patients with CAD had significantly different serum hs-CRP levels. ((1.48±0.0.10 vs 3.33±0.40 mg/l, p<0.01), and between control and T2DM subjects (0.75±0.43 vs 1.48±0.10 mg/l, p<0.01).

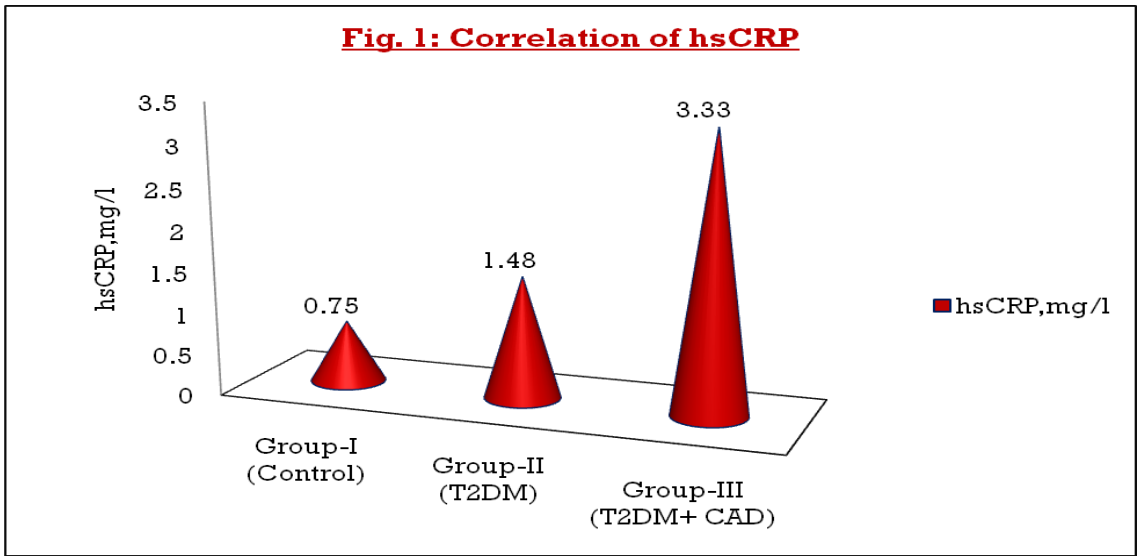

Fig.1 illustrates the levels of hs-CRP in Control subjects, T2DM patients and T2DM patients diagnosed with CAD.

Correlation Coefficient of Hs-CRP

Linear relationship between hs-CRP with anthropometric and biochemical characteristics of this study are given as under;

Table.2: Linear relationship between hs-CRP and other attributes

Description	r Value	p value
Age,Years	-0.011	0.934
BMI,kg/m2	0.768**	0.000
SBP,mmHg	0.858**	0.000
DBP,mmHg	0.697**	0.000
TGL,mg/dl	0.660**	0.000
TC,mg/dl	0.454**	0.000
HDL,mg/dl	**-0.566****	0.000
TC/HDL	0.582**	0.000
LDL,mg/dl	0.664**	0.000

**The significance level for correlation is 0.01 (2-tailed).

*At a 2-tailed significance threshold of 0.05, correlation is significant.

Pearson correlation was used for the statistical analysis.

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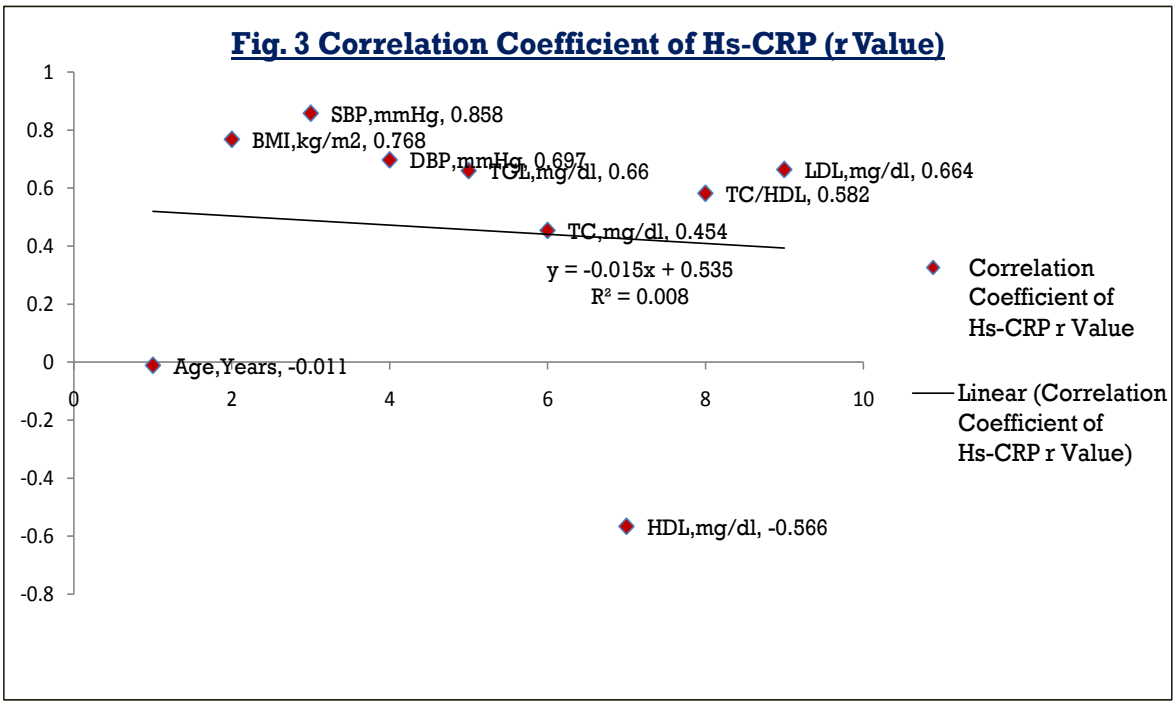

Fig.2 illustrates the Linear relationship between hs-CRP and other attributes.

DISCUSSION

Analyses from large-scale clinical trials have used a hs-CRP cut point of 2 mg/l for defining increased CAD risk [13,14] . Various evidences have implicated CRP as a mediator of endothelial dysfunction[15]. By releasing numerous cytokines hs-CRP can directly promote monocyte activation[16]. hs-CRP levels increases the conversion of monocytes into atheromatous plaque and induces endothelial dysfunction by suppressing basal and induced nitric oxide release. The hs-CRP found to increase the expression of vascular endothelial plasminogen activator inhibitor-1 (PAI-1) and other adhesion molecules and alter LDL uptake by macrophage. Several studies including Framingham study have suggested that hs-CRP has sufficient risk prediction value for atherosclerosis[17,18,19]. Relatively high levels of hs-CRP in T2DM patients have been found to be predictive of an increased risk of a future heart attack, stroke, sudden cardiac death, and/or peripheral arterial disease, even when cholesterol levels are within an acceptable range.

With the publication of the Physician's Health Study (PHS) in 1997, there was a hypothesis alteration in the use of hs-CRP as a marker of CAD risk. People with higher hs-CRP values have the highest risk of cardiovascular disease and those with lower values have less risk. Specifically, individuals who have hs-CRP results at the high end of the normal range have 1.5 to 4 times the risk of developing Coronary Artery Disease as those with hs-CRP values at the low end of the normal range. This study states that the levels of hs-CRP ranges between 0.3 and less than 1.0 among control subjects, 1.0 to 2.0 among T2DM subjects, more than 3.0 in T2DM patients diagnosed with CAD. This study results positively correlates with the study of American Heart Association. From this we can conclude that, the

risk of developing CAD will be lower, where the levels of hs-CRP level under 1.0 mg/L, moderate risk for those have the hs-CRP levels between 1.0 and 3.0 mg/L,& and high for those who have the hs-CRP levels above 3.0 mg/L.

These findings further establish hs-CRP as a significant risk factor for the emergence of clinical manifestations of CAD by indicating that the elevated risk of future coronary events seen in patients with elevated serum hs-CRP is directly related to the elevated number of vulnerable plaques prone to rupture. Serum hs-CRP levels that are elevated in CAD and T2DM patients may be a sign of continuous atheromatous plaque inflammation, which could make the plaque more vulnerable and cause the development of unstable syndromes. According to the association between serum hs-CRP levels and unfavourable coronary artery disease events, atheromatous plaque stability is not necessarily indicated by clinical "stability" [20].

Our research supports Schottker et al.'s (2013) findings that hs-CRP levels are related to cardiovascular events and that hs-CRP level and LDL level were therapeutically effective for predicting CAD occurrences. Our results further demonstrate that hs-CRP is clinically helpful for the prediction of macro and micro vascular events in CAD. In T2DM patients, circulating Adiponectin levels were found to be inversely linked with hs-CRP levels, according to Shetty CK et al. (2004). Von Eynatten et al. (2006) demonstrated that hs-CRP levels in CAD patients were not statistically significant, in contrast to our findings. Our investigation demonstrated a relationship between hs-CRP and the degree or severity of CAD. There is debate concerning the relationship between hs-CRP and the severity or extent of CAD. These results do not agree with those presented by Azar et al. in 2000[21].The study by Pradhan AD et al., 2001 also shows that patients with elevated basal levels of CRP are at an increased risk of diabetes, hypertension, and cardiovascular disease. This finding is consistent with our own study.

CONCLUSION

These findings highlight the relationship between inflammation and hs-CRP, making it a significant inflammatory biomarker. When compared to the normal and T2DM groups, hs-CRP levels were considerably higher in CAD with T2DM patients. In T2DM with CAD patients, hs-CRP levels were significantly lower than in normal and T2DM patients, suggesting that hs-CRP may be an established superior bio marker for monitoring cardiovascular events.

The use of hs-CRP in predicting the risk of CAD is constrained, nonetheless, due to its lack of specificity. Given that losing weight is crucial for reducing inflammation, methods of weight loss promotion, in particular engaging in healthy physical activity, can induce the anti-inflammatory effects of exercise and can lessen the inflammatory effects that may be linked to a drop in hs-CRP levels, supporting the idea that altering one's lifestyle can unquestionably lower inflammation and the risk of developing coronary artery disease (CAD).

Limitations

The hs-CRP evaluation has a number of limitations that must be taken into account. Inflammatory indicators have been proven to predict total mortality as well as cardiovascular events. They are non-specific, rise with acute infection or trauma.

Clinical efficacy may therefore be constrained by the requirement to avoid hs-CRP testing during illness or trauma and among those with known systemic inflammatory disorders. However, as a result of these factors, epidemiological studies have underestimated the genuine predictive value of hs-CRP.

It's unclear how hs-CRP testing is used to different T2DM populations. However, although the cost efficacy of hs-CRP testing has not been formally assessed, it is inexpensive and is likely to be so, especially when compared to methods like magnetic resonance imaging (MRI) or electron beam computed tomography (EBCT).

After acute ischemia, hs-CRP levels can significantly increase, making it challenging to determine an individual's underlying baseline level. This impact may lead to misdiagnosis. Additionally, among people who have recently experienced an acute infarction, measurements of ventricular function and infarct size are likely to have a much higher predictive value. Therefore, to ascertain whether hsCRP evaluation has value in secondary prevention of CAD rather than generalising results from primary prevention, carefully controlled studies of post-infarction patients that include information about ventricular function and other significant prognostic factors are required.

Summary

Measurement of hs-CRP may offer a cutting-edge tool for identifying people at high risk of developing CAD because it plays a significant role in atherothrombosis. According to a number of sizable prospective studies, both men and women who appear to be in good health can use hs-CRP as a strong independent predictor of future myocardial infarction and stroke. This study also supports the notion that an increase in hs-CRP will result in atheromatous plaque and CAD.

Though many inflammatory markers available for predicting the risk of CAD, hs-CRP may be treated as proven bio marker for prediction of risk of CAD among T2DM. This study demonstrates that the use of hs-CRP in routine clinical practise may have a function as an adjuvant to global risk assessment in the primary prevention of cardiovascular disease, despite the limitations of inflammatory screening that still exist.

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Ethical Clearance: Taken

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