Original Research Article

A study on high sensitivity C-reactive protein as a predictor of atherosclerotic coronary artery disease in diabetes mellitus

P. Dharmarajan[1], I. Periyandavar[2*], T. Jaya Packiam[3], Govarthanan[4]

[1]Director, Institute of Diabetology, Madras Medical College, Chennai, Tamil Nadu, India
[2]Senior Consultant in Diabetology, TN Govt. Multi Super- Speciality Hospital, and Professor of Biochemistry, Institute of Diabetology Madras Medical College, Chennai, Tamil Nadu, India
[3]Assistant Professor, Institute of Diabetology, Madras Medical College, Chennai, Tamil Nadu, India
[4]Assistant Professor, Institute of Diabetology, Madras Medical College, Chennai, Tamil Nadu, India
[*]Corresponding author email: **periyandavardr@gmail.com**

International Archives of Integrated Medicine, Vol. 7, Issue 4, April, 2020.
Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 28-03-2020 **Accepted on:** 03-04-2020
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: P. Dharmarajan, I. Periyandavar, T. Jaya Packiam, Govarthanan. A study on high sensitivity C-reactive protein as a predictor of atherosclerotic coronary artery disease in diabetes mellitus. IAIM, 2020; 7(4): 56-62.

Abstract

Background: Diabetes mellitus is characterized by increased circulating plasma Glucose concentrations associated with abnormal metabolism of Carbohydrate, protein, fat and a variety of microvascular and macro Vascular complications. Cardiovascular disease is one of the leading causes of mortality and morbidity in patients with diabetes. It results from the sequelae of Atherosclerotic coronary artery disease and the most common Manifestations are acute myocardial infarction, angina, heart failure, and Sudden death. Atherosclerosis, the underlying cause of coronary artery disease Starts early in life and progresses slowly usually for decades. A well-known marker of inflammation, namely C- reactive protein with the recent high Sensitivity assays (hs- CRP) can detect inflammation associated with Atherosclerosis at an early stage.

Aim of the study: The present study was aimed at the evaluation of high sensitivity C- reactive protein as a marker for atherosclerosis. High sensitivity C- reactive protein assay in patients with diabetes mellitus associated with and without coronary artery disease is performed and the results are correlated with lipid profile.

Materials and methods: The Study Population was derived from the patients attending the Outpatient Department of Institute of Diabetology, Madras Medical College, in the year 2018. A hundred subjects were chosen for the study. All of them were males of age group 45 years - 70 years

and informed consent were obtained from all of them. Fifty subjects, who were diagnosed to be diabetic, as per the criteria of American diabetes association and WHO 2004, with no evidence of atherosclerotic coronary artery disease in Electrocardiogram, formed the control group.

Results: In the present study, the mean value of hs- CRP showed a significant increase in the study group compared to the control group. The results of the present study showed a rise up to 2 fold in the levels of hs- CRP. The study group had low-density lipoprotein levels only slightly above the cutoff range. Rather than changes in the concentration of the low-density lipoprotein, functional defects of low-density lipoprotein, namely formation of small, dense low-density lipoprotein had occurred in the study group. These molecules are pro-atherogenic. They had predisposed the study group population to their present disease condition. Hence the patients taken as a control group for the present study based on normal ECG findings had to be evaluated by other tests to assess the cardiac function and must be followed up.

Conclusion: High Sensitivity C- reactive protein is useful as a marker for atherosclerotic Coronary artery disease in Diabetes mellitus. Any patient with increased High Sensitivity C- reactive protein levels must be investigated for atherosclerotic Coronary artery disease.

Key words

High sensitivity C- reactive protein, Dyslipidemia, ECG, Atherosclerosis.

Introduction

Diabetes mellitus is characterized by increased circulating plasma glucose concentrations associated with abnormal metabolism of carbohydrate, protein, fat and a variety of microvascular and macrovascular complications [1]. The prevalence of both type 1 and type 2 diabetes has risen dramatically over the past two decades and is expected to rise phenomenally in the future due to decreased physical activity and obesity. It is predicted that type 2 diabetes will be increased to pandemic proportions in a few decades in the Indian population [2]. Cardiovascular disease is one of the leading causes of mortality and morbidity in patients with diabetes. It results from the sequelae of atherosclerotic coronary artery disease and the most common manifestations are acute myocardial infarction, angina, heart failure, and sudden death [3]. Atherosclerosis, the underlying cause of coronary artery disease starts early in life and progresses slowly usually for decades. It is not simply a disease of lipid deposition, but of an endothelial injury leading to endothelial dysfunction and local inflammatory changes which predispose to the formation of atherosclerotic plaque [4]. These changes play a pivotal role in atherothrombotic disease progression. Diabetes is an independent risk factor for coronary artery disease and often is not associated with typical anginal symptoms, it becomes difficult to diagnose the process of atherosclerosis at an early stage [5]. The inflammatory changes found to be associated with an atherosclerotic disease process can be used for its identification [6]. A well-known marker of inflammation, namely C-reactive protein with the recent high sensitivity assays (hs- CRP) can detect inflammation associated with atherosclerosis at an early stage [7].

Materials and methods

The study population was derived from the patients attending the Outpatient Department of Institute of Diabetology Madras Medical College, in the year 2018. A hundred subjects were chosen for the study. All of them were males of age group 45 years - 70 years and informed consent were obtained from all of them. Fifty subjects, who were diagnosed to be diabetic, as per the criteria of American diabetes association 2018 and WHO 2004, with no evidence of atherosclerotic coronary artery disease in Electrocardiogram, formed the control group.

Inclusion criteria: Subjects diagnosed to be diabetic with any one of the following criteria - Symptoms of diabetes plus random plasma glucose concentration of ≥200 mg/dL, (or) Fasting (defined as no caloric intake for at least 8 hours) plasma glucose ≥ 126 mg/dL, (or) Two hours of plasma glucose ≥200 mg/dL during an oral glucose tolerance test. Presence of atherosclerotic coronary artery disease evidenced by Electrocardiogram changes like Abnormal ST-T wave changes (ST-segment elevation or depression or deep symmetrical T wave inversion) and formation of new Q waves.

Exclusion criteria: Presence of hypertension, defined by resting systolic blood pressure > 140 mmHg and diastolic blood pressure > 90 mm Hg, presence of Obesity, defined by body mass index more than 30 kg/m^2, presence of malignancy and concomitant systemic diseases like rheumatic disease, chronic liver disease, renal disorder, and sepsis, critically ill patients, smokers, and alcoholics, ongoing infectious diseases.

Blood collection: 5ml of blood samples were collected by venepuncture with strict aseptic precaution after an overnight fast for at least 12 - 14 hours. 1 ml of the sample was aliquoted into a test tube containing EDTA and Sodium fluoride mixture (0.5 mg + 1mg). The sample was centrifuged and plasma separated and the analysis for glucose was done immediately. Remaining 4 mL of the sample was allowed to clot and the serum was separated after centrifugation and the analysis of total cholesterol, triglycerides, high-density lipoprotein was done immediately and 0.5 mL of serum was stored at -20 ˚C for analysis of high sensitivity C- reactive protein.

Statistical analysis

Statistical analysis of the data obtained from the study was done using the 'z' test or 'normal' test to compare the mean values of two groups of participants. The chi-square test was used to compare the prevalence between the two groups. The calculations were done for a 5% level of significance (P = 0.05).

Results

A total of 100 patients were included in the present study. Out of the 100, 50 were study group (Diabetes mellitus with Atherosclerotic coronary artery disease) and the other 50 were controls (Diabetes mellitus without Atherosclerotic coronary artery disease).

Male patient in the age group 45 years - 70 years were taken for the study. Both the Study and control group patients were age-matched. The mean age of the study group was 54.94 and the mean age of the control group was 52.88 (**Table – 1**). Comparison of the biochemical parameters in the study and control group was as per **Table – 2**.

The control group data obtained in the present study showed that 35 out of 50 patients have hs-CRP levels in the range of 1-3 mg/L which is included in the moderate cardiovascular risk group by AHA/ CDC panel (**Graph – 1**).

Table - 1: Age distribution among the study and control group.

Group	N	Mean	Standard Deviation	Student independent t-test
Control	50	52.88	6.08	t=1.67 p=0.09
Study	50	54.94	6.28	Not Significant

Discussion

Epidemiological and clinical studies have shown a strong relationship between markers of inflammation and the risk of future cardiovascular events. Inflammation can be detected locally by temperature and pH changes. It can be detected systemically by measurement of inflammatory markers [8]. The markers of inflammation reported so far in atherosclerosis include high sensitivity C reactive protein,

interleukin-6, serum amyloid A, tumor necrosis factor-alpha, soluble intercellular adhesion molecule-1, macrophage inhibitory cytokine-1, p- Selectin, CD40 ligand. Among these various markers, high sensitivity C- reactive protein is widely studied experimentally and is easy to estimate in the laboratory [9].

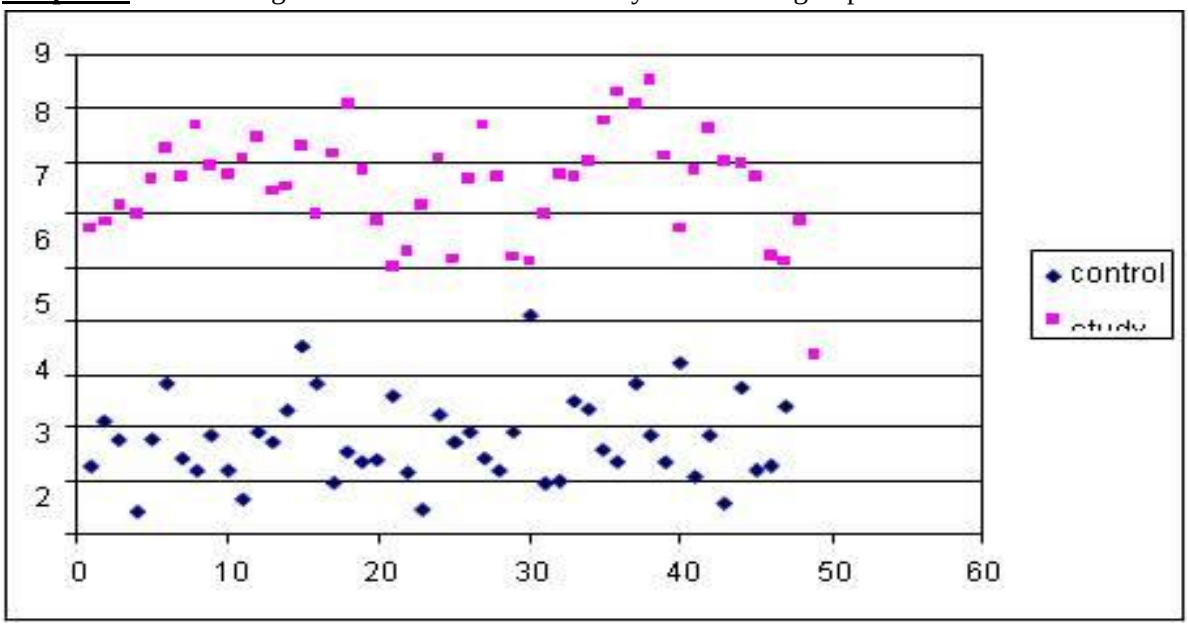
Table – 2: Comparison of the biochemical parameters in the study and control group.

Parameter	**Group**	**Mean**	**Standard Deviation**	**Student Independent t test**
Plasma Glucose	Control	120.66	37.98	t=0.67
	Study	125.50	33.90	p=0.50
				Not Significant
Total Cholesterol	Control	178.84	42.91	t=1.42
	Study	190.42	38.16	p=0.16
				Not Significant
Triacylglycerol	Control	128.90	66.39	t=2.08
	Study	158.76	17.16	p=0.04
				Significant
High density	Control	38.08	9.27	t=2.74
lipoprotein	Study	33.30	8.12	p=0.01
				Significant
Low density	Control	107.54	38.55	t=3.25
lipoprotein	Study	131.34	34.39	p=0.05
				Significant
Very Low density	Control	25.68	13.28	t=2.01
lipoprotein	Study	29.78	3.41	p=0.05
				Significant
Total Cholesterol/	Control	4.94	1.43	t=3.49
High density	Study	5.91	1.34	p=0.001
lipoprotein				Significant
Low density	Control	2.99	1.21	t=4.43
Lipoprotein / High	Study	4.04	1.14	p=0.001
density lipoprotein				Significant
High Sensitivity C-	Control	1.74	0.78	t=26.15
reactive protein	Study	6.51	1.02	p=0.001
				Significant

It has been shown in the Honolulu heart study, that the measurement of high sensitivity C-reactive protein can be used to identify the risk of coronary artery disease at a very early stage. The traditional risk factors used in the prediction of atherosclerosis are low-density lipoprotein cholesterol, triacylglycerol, total cholesterol, LDL/HDL ratio, while high-density lipoprotein is a marker of anti-atherogenic potential in an individual. But the efficacy of these traditional risk factors is questionable in their ability to identify all the individuals at an increased risk. This has been shown in a 2003 study of more than 120,000 patients that approximately 20

percent of all coronary events occurred in the absence of any major risk factors like hyperlipidemia, hypertension, diabetes, and smoking [10]. In the present study, the mean value of hs- CRP showed a significant increase in the study group compared to the control group. The results of the present study show a rise up to 2 fold in the levels of hs- CRP, which is consistent with the data given by Inoguchi T who documented a 1.5 to 7 fold increase of hs- CRP in patients with symptomatic atherosclerosis [11]. This data coincides with the results of James T, et al. [12]. They have reported that hs-CRP is an independent biomarker for predicting coronary artery disease in the diabetic population. In their follow up study the incidence of fatal cardiovascular events was much higher in patients who had hs- CRP in the levels of moderate to high cardiovascular risk group. Hence the patients taken as a control group for the present study based on normal ECG findings have to be evaluated by other tests to assess the cardiac function and must be followed up [12].

Graph – 1: Scatter diagram of hs- CRP levels in study and control group.



As both the groups in the present study are diabetics, the mean differences in the values of fasting plasma glucose concentration between the control and study groups are not significant. The values also reveal that the present study group subjects are well-controlled diabetics. The mean value of low-density lipoprotein cholesterol in the study group is 131.32. The LDL goal for the patients with multiple risk factors (i.e.) age > 45 years, male gender in the present study, according to National Cholesterol Education Program (NCEP) expert committee is less than 130, to reduce the risk of a major coronary event [13]. The study group has low-density lipoprotein levels only slightly above the cutoff range. Rather than changes in the concentration of the low-density lipoprotein, functional defects of low-density lipoprotein, namely formation of small, dense low-density lipoprotein would have occurred in the study group. These molecules are pro-atherogenic [14]. They could have predisposed the study group population to their present disease condition. The Adult Treatment Panel III (ATP III) classifies less than 40mg/dL HDL Cholesterol as low and more than 60 mg/dL HDL. Cholesterol is as high [15]. The mean value of HDL- C in the present study group is 33.30, which is classified as low HDL- C. The LDL/HDL ratio, which is an indicator of atherogenic potential, is significantly raised in the study group, and it is due to a decrease in the HDL levels, which could have predisposed the study group population to the coronary artery disease [16]. Moreover, functional defects in the

HDL-C are more common in diabetics leading to a decreased capacity to prevent the oxidation of LDL Cholesterol, promoting atherosclerosis. The mean value of the Total Cholesterol/ HDL-C ratio in the study group is 5.91 [17]. Libby P, in his study, has reported a Total Cholesterol/ HDL-C ratio of more than 5.5 in men and 5.9 in women, strongly correlate with incident cardiovascular disease. Since the value of Total Cholesterol/ HDL-C ratio is one of the strongest predictors of cardiovascular risk, the high value of Total Cholesterol/ HDL-C ratio in the present study is consistent with the previous studies [18]. As all the patients taken for the study were diabetics, the mean difference between the values of total Cholesterol in the control and study groups did not show any statistical significance. The mean value of triacylglycerol in the study group is 158.90, which is more than the cutoff proposed by ATP III as 150 mg/dL. Hence the changes in the lipoprotein concentration with its functional abnormalities due to diabetes could have predisposed to coronary artery disease. It was found that most of the patients taken for the study were aware of the dietary control of the lipids and exercise for their ailment [19]. This is probably one reason for the lipid levels in the study group being very close to the control. The difference in the age-matched study group did not show a uniform significance in any of the measured parameters except hs-CRP [20]. This is probably because of the small number of patients taken for the study. The prognostic additive effect of hs- CRP to the lipid screen is very useful in the diabetic population because the relative risk in these patients can be calculated and the patients on moderate to the high-risk group can be diagnosed earlier because most diabetic individuals do not exhibit classical anginal symptoms [21].

Conclusion

Our study shows that CRP is an independent risk factor for CHD mortality in patients with type 2 diabetes. This suggests that inflammation plays an important role in fatal CHD events also among this high-risk population. This might partly explain why statins and aspirin, in addition to their LDL-lowering and antiplatelet effects, have had favorable effects in studies on the prevention of CHD events in patients with type 2 diabetes.

References

1. Assert R, Scherk G, Bumbure A, et al. Regulation of Protein kinase C by short term hyperglycemia in human platelets in vivo and in vitro. Diabetologia, 2001; 44: 188-195.
2. Beckman JA, Creager MA, Libby P. Diabetes and Atherosclerosis: Epidemiology, Pathophysiology, and Management. JAMA, 2002; 287: 2570-2581.
3. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature, 2001; 414: 813-820.
4. Duel PB, Oram JF, Bierman EL, et al. Non- enzymatic glycosylation of HDL resulting in inhibition of high-affinity binding to cultured human fibroblasts. Diabetes, 1990; 39: 1257-63.
5. Executive summary of the third report of the national cholesterol education program (NCEP). Expert panel on the detection, evaluation, and treatment of high blood cholesterol in adults. (Adult treatment panel III). JAMA, 2001; 285: 2486.
6. Fuster V. Epidemic of Cardiovascular disease and Stroke. Circulation, 1999; 99: 1132-1137.
7. Geng Y-J, Libby P. Progression of atheroma: a struggle between death and procreation. Arterioscler Thromb Vasc Biol., 2002; 22: 1370.
8. Haffner SM, Lehto S, Ronnemaa T, et al. Mortality from Coronary heart disease in subjects with type 2 diabetes and non-diabetic subjects with or without myocardial infarction. N Engl J Med., 1998; 339: 229- 234.
9. Howard G, O' Leary DH, Zaccaro D, et al. Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study

(IRAS) Investigators. Circulation, 1996; 93: 1809-1817.

10. Hu FB, Stampfer MJ, Solomon CG, et al. The impact of Diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow up. Arch Intern Med., 2001; 161: 1717-1723.
11. Inoguchi T, Li P, Umeda F, et al. High glucose levels and Free fatty acids stimulate ROS production through protein kinase C- dependent activation of NADPH oxidase in cultured vascular cells. Diabetes, 2000; 49: 1939-1945.
12. James T. Willerson, Paul M Ridker. Inflammation as a cardiovascular risk factor. Circulation, 2004; 109: II 2- II 10.
13. John F. Golding. Smoking. Baum's Textbook of Pulmonary Diseases. Editors- James D. Crapo, Joel Karlinsky. 7th edition, Lippincott Williams and Wilkins Publishers, 2004, p. 215.
14. Koya D, King GL, Sheetz MJ, et al: Protein kinase C activation and the development of diabetic complications. Diabetes, 1998; 47: 859-866.
15. Lee R, Libby P, Koenig W, et al. The unstable atheroma. Arterioscler Thromb Vasc Biol., 1997; 17: 1859.
16. Lempiainen P, Mykkanen L, Pyorala K, et al. Insulin resistance syndrome predicts coronary heart disease events in elderly non- diabetic men. Circulation, 1999; 100: 123-128.
17. Li Y, Woo V, Bose R, et al. Platelet hyperactivity and abnormal Calcium homeostasis in diabetes mellitus. Am J Physio., 2001; 280: H1480 – H1489.
18. Libby P, Buring JE, Xu DY, et al. Inflammation and atherosclerosis. Circulation, 2002; 105: 1135.
19. Lopes-Virella MF, Klein RL, Lyons TJ, et al. Glycosylation of IDL enhances cholesteryl ester synthesis in human – monocyte-derived macrophages. Diabetes, 1998; 37: 350-357.
20. Miettinen H, Lehto S, Salomaa V, et al. Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA myocardial infarction register study group. Diabetes care, 1998; 21: 69-75.
21. Miller M, Zhan M, Havas S, et al. High attributable risk of elevated C- reactive protein level to conventional risk factors for coronary heart disease. Arch Intern Med., 2005; 165: 2063-2068.