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Research article

SERUM HIGH SENSITIVE C-REACTIVE PROTEIN AND ANTICARDIOLIPIN ANTIBODY LEVEL IN CAD PATIENTS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS.

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ABSTRACT: The increased incidence of coronary artery disease (CAD) in Type 2 Diabetes mellitus is not fully explained by the conventional risk factors. Our aim was to determine the association of biomarkers, high sensitive CRP and anticardiolipin antibody (acL) with severity of coronary artery disease in patients with and without type 2 DM. In our study, hsCRP level was significantly high in CAD with DM and found to be positively correlated with severity ($p<0.01$) while anticardiolipin antibody does not show any significant change among the two groups. Our study concluded that increased risk of CAD in type 2 DM patients is not only because of dyslipidemia but inflammatory events also play a major role. hsCRP was found to be a valuable predictor for CAD in type 2 DM.

Key words: Coronary artery disease, Atherosclerosis, anticardiolipin antibody, high sensitive C-reactive protein.

INTRODUCTION

Coronary artery disease (CAD) is one of the major prevailing non communicable cause of death and disability in the Indian Subcontinent. (Borch et al.,1987)It is caused by atherosclerosis, an accumulation of fatty materials on the inner linings of arteries. The initiation and progression of atherosclerosis involves inflammatory and immunological mechanisms. All manifestations of CAD are substantially more in diabetic patients than non diabetic individuals. But various studies have indicated that excess risk for macro vascular complications in type 2 diabetes mellitus cannot be completely explained by the traditional risk factors. Use of traditional cardiovascular risk factors is imprecise and predicts less than one half of the cardiovascular events. Hence an outlook on novel risk factors is required for the evaluation of coronary artery disease.

Anticardiolipin (acL) antibodies are autoantibodies found to play an important role in atherosclerosis .They are one of the most common acquired defects causing thrombosis. (Bick RL, 2001). These antibodies have been shown to possess proinflammatory (Vaarala O., 1998), and procoagulant (Martinuzzo et al., 1993) properties. C-reactive protein (CRP), an inflammatory marker, is a novel and evolving biomarker for the extend and severity of atherosclerotic lesion. Inflammatory events occurred due to chronic alteration related to insulin resistance, predisposes people to atherosclerosis. It provides a useful predictive indicator for subsequent cardiovascular events and response to treatment. CRP is also considered as an effective marker to track progress of cardiovascular disease. So the present study was designed to compare the plasma levels of anticardiolipin antibody and high sensitive CRP with other lipid variables of angiographically defined CAD patients with and without type 2 diabetes mellitus (type 2 DM) and to assess their power as a marker of CAD in a sample of South Indian population.

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MATERIALS AND METHODS

This study was conducted at the department of biochemistry and cardiology of Vinayaka Missions Hospital, Salem between August 2009 and July 2010. Study group consisted of one hundred and nine individuals with established CAD in the age group of 40-70 years who had undergone coronary angiography and diagnosed with coronary artery disease including single vessel, double vessel and triple vessel and seventy one healthy individuals matched for age, and sex. The subjects were grouped into CAD patients with type 2 DM (CADWDM) (n=57)and CAD patients without type 2 DM (CADWNDM)(n=52) and normal healthy group (n=71).From each patient, their medical history was obtained through a structured questionnaire and an informed consent was obtained. The inclusion criteria include patients with established coronary artery disease including single vessel, double vessel and triple vessel. Those who had undergone a treadmill test positive for inducible ischemia, patients with history of essential hypertension, coronary artery disease patients with essential hypertension who had border line rise in fasting blood glucose, and patients with recent onset of diabetes. type 2 DM was diagnosed according to the WHO criteria. Patients excluded were those diagnosed to have coronary artery disease with atrial fibrillation or pacemaker, history of congestive heart failure, history of stroke, transient ischemia or carotid surgery, history of coronary artery bypass graft surgery or percutaneous, transluminal coronary angioplasty, history of intermittent claudication or peripheral vascular surgery. Venous blood sample was collected after an overnight fast of 12 hours and the serum was used for the estimation of fasting blood glucose (FBG) by enzymatic GOD-POD method, cholesterol by enzymatic 'CHOP-PAP' method, triglyceride (TG)by enzymatic GPO-POD method and high density lipoprotein cholesterol (HDL-C) by direct enzymatic colorimetric method. LDL-C and VLDL-C were calculated using the Friedewald's formula (Friedewald's W T, et.al.,1972). Anticardiolipin antibody- IgG was measured by immunoassay method and high sensitive CRP was measured by particle enhanced immunoturbidimetric test (Euro diagnostic systems).

STATISTICAL ANALYSIS:

All statistical analysis were performed using the SPSS software (Statistical Package for the Social Sciences, version 16.0 SPSS Inc. Chicago ,III USA.) Quantitative variables were demonstrated as Mean±SD .Statistical analysis has been done using 'Chi square test. .Association between the factors were analyzed using Pearson correlation.

RESULTS

In the 57 subjects of CADWDM, no subjects had anticardiolipin antibody titer > 15 GPL and 52 CAD WN DM. Our report shows that diabetic patients with CAD have higher CRP levels than CAD patients without DM.

Table 1: Grading of CAD Patients

GRADE I	<50% stenosis with single vessel disease .(mild)
GRADE II	50-74% stenosis with double vessel disease. (moderate)
GRADE III	75-100% stenosis with triple vessel disease. (severe).

Based on severity of CAD the subjects were classified into Grade I,Grade II and Grade III.

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Table 2: Demographic and Biochemical Parameters of Study

PARAMETERS	CONTROL GROUP (N=71) GROUP I	CAD WN T2 DM (N=52)-GROUP II	CAD W T2 DM .(N=57)-GROUP III
Age (years)	52.42±6.74	55.4 ±5.657	62 ±9.5
BMI (kg/m2)	20.05±0.95	23.82 ± 3.359	27.05 ± 0.33[a]
SBP mm of Hg	116.14±10.2	140.9 ± 16.97[b]	147.5 ± 28.28[a,b]
DBP mm of Hg	71.14±7.82	88.92±14.14	95.78 ± 21.21[b]
FBS (mg/dl)	85± 6.21	85.65 ± 5.657	147.05 ± 30.4[a,b]
PPBS (mg/dl)	101.81±5.105	109.4±7.071	403 ± 53.7[a,b]
Insulin (µIU/ml)	4.52 ± 3.61	8.76 ± 5.37[b]	22.93 ± 3.25 [a, b]
HOMA - IR	1.77±0.33	1.8± 0.445	9.7 ± 1.1 [a,b]

Data are presented as the means ± SD. Datas were statistically analyzed by students t test [a] Significantly different from group I and [b] Significantly different from group II ($p < 0.05$)

Study subjects were 39% control subjects, 29% coronary artery disease patients without type 2 DM and 32 % were CAD patients with type 2 DM. The study subjects were of the age group 40-75 years. The mean age of onset of CAD in the group with type 2 DM was 50 ± 4.5 when compared to 55±5 in CAD without DM..The DBP was not statistically significant in CAD with type 2 DM when compared to CAD without DM. Statistically significant increase in BMI, FBS, PPBS and insulin resistance was observed in Group III when compared to the Group II and Group I.

Table 3: Severity of CAD among CAD Patients with and Without Type 2 DM

Description	Severity of CAD		
	Grade I <50% ,1	Grade II 50-75%, 2	Grade III 76-100%,3
CAD without DM	24(46%)	15(29%)	13(25%)
CAD with DM	9(16%)	12(21%)	36(63%)

Analysis on frequency of distribution has revealed that severity was significantly high in CAD with DM. The percentage of patients with severe coronary artery disease was 63% in CAD with DM compared to 3 % in CAD without DM.

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Table 4: Lipid Parameters of Study Subjects.

Description	CONTROL (N= 71)GROUP I	CAD WN T2 DM (N=52) GROUP II	CAD W T2 DM .(N=57) GROUP III
Total cholesterol (mg/dl)	169.7 ±4.2	182.2 ± 8.485	197.49 ± 7.77
Serum Triglycerides mg/dl)	I21.35±11.28	131.6 ± 6.4	157.5 ± 8.5a
HDL cholesterol (mg/dl)	41.48 ± 5.59	38.4 ± 2.07	35.05 ± 1.44a
LDL cholesterol (mg/dl)	104.48±33.58	129.50 ± 8.8	144.25 ± 8.2a
VLDL (mg/dl)	24.27±2.26	26.2±3.01	28.94 ± 9.617

Data are presented as the means ± SD. Datas were statistically analyzed by students t test. [a] Significantly different from group I ($p < 0.05$)

The level of Total cholesterol, Triglycerides ,HDL-C, LDL-C and VLDL among the control, CAD with diabetes mellitus and CAD without diabetes mellitus were analysed.

Table 5: Correlation of Lipid Parameters and Severity of CAD

Lipid Parameters	Severity of CAD	
	r value	p value
Total cholesterol (mg/dl)	-.131	.079
Serum Triglycerides (mg/dl)	**.244**	**.011***
HDL cholesterol (mg/dl)	.053	.477
LDL cholesterol (mg/dl)	.099	.306
VLDL (mg/dl)	-.048	.523

Correlation analysis had shown that only triglyceride value was significant at $p<0.01$ when compared to other lipid parameters.

Table V depicts the correlation of lipid parameters and severity of coronary artery disease. The triglyceride level was found to have a positive correlation ($p<0.01$) when compared to other lipid parameters.

Table 6: Risk Parameters of Study Subjects.

PARAMETERS	CONTROL (N= 71) GROUP I	CAD WN DM (N=52)GROUP II	CAD W DM .(N=57)-GROUP III
hsCRP (mg/L)	0.726± 0.173	2.906 ± 0.947a	4.529 ± 0.558a,b
Anticardiolipin Antibody (GPL/ml)	2.356± 1.25	3.69 ±0.141a	3.609 ± 0.131 a

Data are presented as the means ± SD. Datas were statistically analyzed by students t test.[a] Significantly different from group I ($p < 0.05$) [b] Significantly different from group II ($p < 0.05$)

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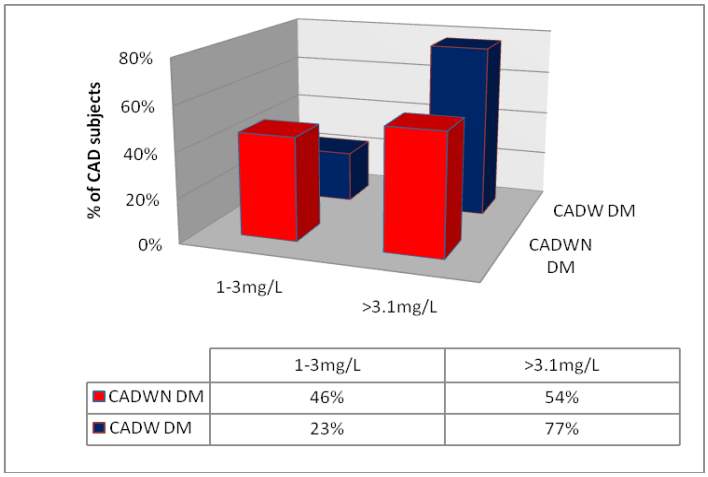
The table shows the distribution of hs CRP and anticardiolipin antibody level in control, CAD with out diabetes and CAD with type 2 diabetes subjects.

Table 7: Distribution of hs CRP among CAD Patients



	1-3mg/L	>3.1mg/L
CADWN DM	46%	54%
CADW DM	23%	77%

Cut off values: Normal level of hsCRP was considered to be present if the level of hs CRP was <3mg/L.

Cut off values: Normal level of hsCRP was considered to be present if the level of hCRP was <3mg/L.

Table 8: hsCRP and severity of CAD.

GROUPS		Grade I	Grade II	Grade III
CADwithoutDM	1-3 mg/L	21	1	2
	>3.1mg/L	3	14	11
CAD with DM	1-3mg/L	6	3	4
	>3.1mg/L	3	9	32

Severity of CAD was found to be significantly high ($p<0.001$) in CAD with diabetes subjects with hsCRP level >3.1 mg/dl. Compared to CAD without diabetic subjects..

Severity was found to be high in CAD subjects with Grade III status and hsCRP level >3.1 mg/L.

DISCUSSION

CAD is a complex, multifactorial disorder. Type 2DM is associated with an increased risk of CAD and is considered to be a model of premature atherosclerosis. Grundy et al has reported that 40-50% of individuals with CAD have type 2 DM. (Grundy.*et al*.,2002).In our study we have observed that the severity of CAD was significantly high in CADWDM, when compared to CADWNDM. Also insulin resistance was found to be significantly high in CADWDM patients when compared to CADWNDM. Triglyceride level was found to be significantly high ($p<0.01$) in Group III compared to Group II when lipid parameters and severity of CAD were analyzed. .Major reason for hypertriglyceridemia, in type 2 DM may be deficient lipoprotein lipase activity, increased cholesteryl ester transfer protein activity; and increased flux of free fatty acids to the liver. Elevated triglycerides are a common and important component of the so-called metabolic syndrome, which predominantly includes obesity, hypertension, hypertriglyceridemia,, and insulin resistance (Gardner *et al* .,1996)..The increase in IR leads to increased BMI as a part of dyslipidemia events. Clinical and epidemiological studies have substantiated an association between inflammation and IR (Ross R., 1999).

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The role of inflammation in the pathogenesis of CVD is well recognized. Inflammation contributes to all phases of atherosclerosis, from fatty streak initiation, growth, and complication of the atherosclerotic plaque to CVD events .Type 2 Diabetes mellitus is now considered as an inflammatory disease and inflammatory process seems to play an important role in the development of diabetes and its late complications. This might be the reason for the significantly high level of acute phase inflammatory marker CRP observed in the CAD patients with DM when compared to other groups. American Heart Association, recommends that, the hsCRP levels of 1 mg/L constitute low risk, 1-3 mg/L as average risk and >3 mg/L as high risk group for assessment of cardiovascular risk.The result of our study showed mean levels of 4.529 ± 0.558 mg/L in CAD WDM and 2.906 ± 0.947 mg/L in CADWNDM and 0.726 ±0.173 mg/ L in those without events. In the analysis we have also observed a positive correlation between CRP level and severity of CAD. In coronary artery disease patients with DM 23% had hsCRP value between 1-3mg/L and 77% had hsCRP value >3mg/L .where as in coronary artery disease patients without DM,46% had hsCRP value between 1-3mg/L and 54% had hsCRP value >3mg/L. Further CRP value was found to be > 3.1 in 34 out of 36 subjects with Grade III stenosis in CAD with DM and 11 out of 13 subjects with Grade III stenosis in CAD without DM.C-reactive protein is a primitive acute phase inflammatory protein synthesized in liver in response to the cytokine and Interleukin-6, is also a factor in the development of atherosclerotic plaque. Ridker P M et al has reported that hCRP is involved throughout the atherogenic process, from the initial recruitment of leukocytes to the arterial wall to the eventual rupture of plaque.(Ridker *et al*., 2003).Li et al showed that (Li *et al*.,2004)CRP directly contribute to endothelial dysfunction. Human aortic endothelial cells cultured with CRP showed upregulation of Lectin-like oxidized LDL receptor-1 (LOX-1),. Oxidized LDL binds to, LOX-1 on endothelial cells and generates superoxide anions, decreases nitric oxide production and activates the transcription factor nuclear factor kB (NF-kB). LOX-1 also increases monocyte binding to endothelial cells. These evidences prove that hsCRP not only an inflammatory marker but also an atherogenic molecule(Pradhan *et al.,* 2001, Danesh *et al* .,2004) Our results substantiate this fact and disclose the significance of predictive value of CRP over traditional risk factors for coronary artery diseases. Anticardiolipin (aCL) antibody type of antiphospholipid antibody binds to Cardiolipin in presence of cofactor β2 glycoprotein 1 .These (aCL) antibodies may promotes ischemia and thrombosis through several mechanisms like functional alteration of protein C ,impaired fibrinolysis ,altered anti thrombin level ,inhibition of prostacyclin activity ,platelet aggregability and complement activation. According to hypothesis of Karera ;s and Wermilen aCL facilitate coagulation by inhibiting the release of arachidonic and thus release of prostacyclin decreases and platelet aggregates. Varala O et al showed that the increased risk of thrombolytic and atherosclerotic events seen in patients with SLE and antiphospholipid antibodies may be due to cross reactivity between anticardiolipin and OxLDL (Vaarala O et al., 1993).

The immune complexes involving antiphospholipid antibody may form in the nascent plaque further contributing to inflammatory process .Studies analyzing the association of anticardiolipin antibodies macro and microvascular complications in type 2 DM are scarce. (Macworth., 2004) Triolo et al. reported that IgA and IgG antibodies against cardiolipin, have a role in the pathogenesis and progression of diabetic micro and macrovascular complications by impairing the thrombo resistant property of the vascular endothelium and causing an increase in platelet aggregation (Triolo *et al*., 1989).In our study a significantly high level of aCL antibody was observed in CAD patients with type 2DM when compared to control, but no significant difference was observed when compared to CAD without type 2 DM subjects. There was no significant association between the presence of aCL antibodies and severity of coronary artery disease in type 2 DM patients and without type 2 DM. aCL is found to have a role in initiation of atherogenesis .But our study was done on established CAD patients .There have been three major studies on myocardial infarction in which the aCL antibodies was not appreciably higher in patients than in control subjects, and antibodies were not predictive for subsequent cardiovascular complications.(De Caterina *et al.,*1990 Sletnes *et al.*, 1992)and Phadke *et al.,*1993),All of these studies were performed on survivors of myocardial infarction or in patients with established coronary heart disease , whereas Hamsten et al observed an increased prevalence of patients with elevated anti-cardiolipin (aCL) antibody levels in a highly selected series of young patients with myocardial infarction. (Hamsten *et al.* 1986).

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CONCLUSION

In our study the conventional risk factors failed to explain the severity of CAD . The increased level of hsCRP in CAD with DM patients states that inflammation precedes the thrombolytic events in the development of atherosclerosis. Also development of high sensitive CRP assays has encouraged arguments in favour of the inclusion of routine assessment of the inflammatory status during the determination of CAD risk profile in patients .In our study there was no association between the anticardiolipin antibody level among the CAD patients with and without type 2 DM. .Further studies in a large population and prospective follow up should elucidate the macro and micro complications of anticardiolipin antibody in CAD patients with and without DM.

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