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Association of plasma levels of soluble CD40 ligand with angiographic severity of Coronary Artery Disease in Indian population

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Abstract:

Background: Soluble CD40 ligand (sCD40L) has been shown to have a significant role in pathogenesis of atherosclerosis development and plaque destabilization. The present study was done to investigate the association of sCD40L with angiographic severity of coronary artery disease in patients suffering from acute coronary syndrome (ACS).

Methods: The study group comprised of 75 patients suffering from acute coronary syndrome. Blood sample was collected witnin 24 hours of admission prior to any intervention or angioplasty. Patients were divided into three groups of angiographic vessel score (AVS) according to coronary artery blockage – AVS1, AVS2, AVS3. Serum levels of sCD40L and CRP were measured using an ELISA and Troponin T was estimated by electro chemiluminiscence. Severity of coronary artery disease was evaluated using angiograpic vessel score (AVS).

Results: Out of 75 study patients, 65 (87.5%) were males with mean age of 51.7 years. There was significant association between sCD40L levels and Angiographic vessel score (p=0.001) with sCD40L levels increasing with its severity [AVS-1, AVS-2 and AVS-3; 0.65 ng/dl (2.5) , 0.20 ng/dl(2.66), 3.7 ng/dl(5.99)]. On the other hand, the median hs-CRP levels had no significant relationship with Angiographic vessel score (AVS-1, AVS-2 and AVS-3; 7.42 (5.3), 5.92 (5.9) and 7.19 (6.25) respectively)

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Conclusion: Serum sCD40L levels were found to be significantly associated with angiographic severity in patients suffering from acute coronary syndrome compared to hs-CRP levels.

Keyword: Atherosclerosis; coronary artery disease; angiographic vessel score; soluble CD40 ligand; coronary angiography.

1. INTRODUCTION

CVDs are the number one cause of death globally: more people die annually from CVDs than from any other cause. An estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Low and middle-income countries are disproportionally affected. Over 80% of CVD deaths take place in low- and middle-income countries and occur almost equally in men and women. By 2030, almost 23.6 million people will die from CVDs, mainly from heart disease and stroke.[1]

Inflammatory marker hs-CRP is an acute phase reactant, produced in the liver in response to interleukin IL-6. CRP has been shown to actively participate in both atheromatous lesion formation and plaque disruption by increasing the expression of endothelial adhesion molecules and monocyte chemoattractant protein-1 (MCP-1). It also facilitates native LDL uptake into macrophages, promotes monocyte activation and a procoagulant effect by inducing monocytes to synthesize tissue factor. CRP can also activate the classic pathway of complement activation.

Many non-traditional atherosclerotic risk factors have been evaluated in recent years. Among them, CD40 ligand (CD40L), derived mainly from activated platelets and contributes to the pathophysiology of atherosclerosis and atherothrombosis.[2] CD40 ligand (CD40L) is a 39-kd transmembrane protein and is a member of the TNF family, [3] the gene for CD40L is located on chromosome X (q26.3-q27.1). The expression of this ligand is induced on a variety of cells such as B cells and platelets. It has also been demonstrated that CD40L is expressed on the surface of platelets within seconds of platelet activation and then is cleaved to generate a soluble fragment (soluble CD40L, or sCD40L).[4] The receptor of this ligand is CD40, a type I transmembrane protein, a member of the TNF superfamily, is expressed on monocyte/macrophages, endothelial cells, smooth muscle cells, and platelets.[5] As a consequence of CD40L binding to its receptor, several inflammatory processes are initiated including the release of cytokines and the expression of adhesion molecules.[6] Similarly

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sCD40L is a proinflammatory marker that has been shown to have has autocrine, paracrine, and endocrine activities. It has been known to promote atherosclerosis and plaque instability. It is released after platelet stimulation.[6] Elevated levels of sCD40L have been found in those exhibiting hypercholesterolemia, unstable angina, or acute myocardial infarction. [3] It has strong independent prognostic value in healthy individuals and in patients with acute coronary syndrome (ACS).[7,8] However still there are contentious and uncertain points over the use of this biomarker in clinical cardiology. In this study, we try to explore the potential implications of raised sCD40L as a biomarker in patients with atherosclerotic vascular diseases. And since no study in India has yet correlated sCD40L and severity of atherosclerosis in patients with ACS, therefore, we have conducted this study to evaluate this association.

2. METHODS

2.1 Study participants and ethics

This cross-sectional study was performed in the department of Biochemistry, Maulana Azad Medical College and Department of Cardiology of the associated G.B. Pant Institute of Post Graduate Medical Education & Research (GIPMER), New Delhi. In the present study a total of seventy five individuals who underwent coronary angiography with suspicion of Acute Coronary Syndrome (unstable angina/MI) were inculded. The study group subjects were selected from the patients admitted in CCU of GIPMER.

Inclusion criteria adopted were: patients above 20 years of age having diagnosis of unstable angina or equivalent type of ischemic discomfort with ischemic discomfort occurring at rest or minimal exertion and usually lasting >20 mins or ischemic discomfort being severe and described as frank pain, of new onset (i.e., within 1 month) and associated with one of the following conditons: (1) ECG showing ST-segment elevation or depression > 1.0 mm in two or more contiguous leads; (2) elevated biomarkers of myocardial necrosis (i.e CK-MB > 1 time the upper limit of normal of the local laboratory, or Troponin-T >0.1 ng/ml). Patients with previous history of myocardial infarction or angioplasty were also included only if the myocardial infarction or angioplasty had occured at least 6 months prior to admission. Patients suffering from other heart diseases like valvular heart disorder, cardiomyopathy etc and patients with other diseases like tumors, liver, lung and renal diseases, acute infections and chronic inflammatory diseases like arthritis etc led to exclusion from study. The study was conducted according to the principles stated in Declaration of Helsinki and approved by the Institutional Ethics Committee and informed consent was taken from all participants recruited in this study. All patients had coronary angiogram and/or angioplasty as required. Details of history, clinical course and

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angiography/angioplasty were recorded. All subjects were receiving antiplatelet agents during the study period.

2.*2 Biochemical analysis*

Venous blood samples (5ml) were taken after an overnight fasting under aseptic precautions from antecubital vein. Blood was collected within 24 hours of admission prior to any intervention or angioplasty. Serum total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol were analyzed by enzymatic methods using Randox kits in autoanalyzer olympus AU 400. Serum levels of hs-CRP were determined using immunoassays based on ELISA using commercially available diagnostic kits (Diagnostics Biochem, Canada). A portion of separated plasma was also stored at -80^{o}C for batch analysis. sCD40L was analyzed by immunoassays based on ELISA using commercially available diagnostic kits (Diaclone, France).[9]

2.3 Angiographic analysis

Angiographic vessel scoring[10] was also done for all cases of ACS patients admitted in CCU of G.B. Pant Hospital suspected of having unstable angina or myocardial ischemia (MI). This score estimates the degree of stenosis which correlates with severity of disease. The score was assessed by two experienced independent observers and they were blinded to both the patient's clinical characteristics and sCD40L levels. The score was computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and number of arteries involved. Absent stenosis, Mild stenosis in single artery having more than 50 % stenosis , Moderate stenosis with two arteries having more than 50 % stenosis with no coronary lesions, and Severe stenosis with three arteries having more than 50 % stenosis were assigned vessel scores of 0, 1, 2 and 3 respectively.

2.4 Statistical Analysis

Statistical Analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, Illinosis, USA). Kolmogorov test of normality was performed and the data was presented as median and interquartile range for nonparametric and mean ± SD for parametric tests. The three groups according to vessel score were compared by Kruskal wallis test or Anova test for non parametric or parametric data respectively. The association between parameters was studied using Spearman's correlation coefficient. A p-value <0.05 was considered to be significant.

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Then a receiver operating characteristic (ROC) curve analysis was applied and area under curve (AUC) was calculated to determine the diagnostic efficiency of sCD40L, hs CRP and hs Troponin T. Vessel score groups and 1 and 2 were combined to represent the less severe form of the disease. Vessel score group 3 was taken as the more severe form.

3. RESULTS

3.1 Patient Characteristics

In the present study, out of the total of seventy five individuals who underwent coronary angiography with the suspicion of Acute coronary syndrome (unstable angina/MI), 65 were males and 10 were females with their age ranging from 32 to 70 years. New emerging parameter sCD40L was studied along with influence of conventional risk factors like age, sex, smoking, lipid profile, hs TroponinT, hs CRP, diabetes and hypertension. Baseline characteristics of the 75 patients included in the study are described in Table 1. Sixteen out of total of 75, (12%) patients whose troponin T levels > 0.1 ng/ml or CK-MB levels were higher than the normal limit were diagnosed with acute myocardial infarction (AMI), rest 59 (78%) had unstable angina. Among the subjects smoking was most common conventional risk factor (71.25%) followed by hypertension (38.75%) and alcohol consumption (31.25%) as shown in Table 1. When severity of the disease was assessed according to angiographic vessels score (AVS), it was observed that 27 had single vessel disease, 26 had double vessel disease and 22 suffered from triple vessel disease Table 1.

Table 1: Baseline clinical and demographic characteristics

Characteristics	ACS(n=75)
Age (years)	51.77±10.12
Male (n(%))	65 (87.5%)
Hypertension (n(%))	31 (38.75%)
Diabetes mellitus (n(%))	17 (21.25%)
Current smoking (n(%))	57 (71.25%)
Alcohol consumption (n(%))	25 (31.25%)
Family history (n(%))	18 (22.5%)
Biochemical Parameters	

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TC (mg/dl)	134 (99.2)
TG (mg/dl)	133 (78 .6)
HDLC (mg/dl)	30 (13.6)
LDLC (mg/dl)	85 (45)
Troponin T (ng/ml)	0.04 (.55)
sCD40L (ng/ml)	1.2 (1.6)
Apo A(mg/dl)	102.6±2.8
Apo B(mg/dl)	63.4±2.4
Angiographic vessel score 1 2 3	 27 (30.7%) 28 (40%) 20 (29.3%)

3.2 sCD40L, troponin T, hs CRP, Lipid profile in different groups of angiographic vessel score

Comparisons of all parameters in the three groups according to AVS showed that median levels of sCD40L, Troponin T, total cholesterol, TG, HDL and LDL were significantly higher in triple vessel disease when compared with single vessel and double vessel disease (table 2).

Table 2: Comparison of various biochemical parameters in patients suffering from Acute coronary syndrome on basis of angiographic vessel score

Parameter	Vessel score1 n=27	Vessel score2 n=26	Vessel score3 n=22	P value
sCD40L (ng/dL)	0.65(2.5)	0.20(2.66)	3.7(5.99)	0.001

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TroponinT (ng/ml)*	0.01 (0.09)	0.20(0.66)	0.06 (0.43)	0.035
CRP	7.42 (5.3)	5.92 (5.9)	7.19 (6.25)	0.998
TC (mg/dl)*	119 (45)	126.5 (98.75)	163 (75.25)	0.043
TG (mg/dl)*	96.3 (73)	179 (145)	144.5 (78.25)	0.004
HDLC (mg/dl)	27 (12)	28 (8.25)	37.5 (14.75)	0.005
Apo A1(mg/dl)#	103± 4.77	100.4± 4.488	106.8 ± 6.4	0.867
Apo B100(mg/dl)#	70.9 ±4.1	59.28±3.9	59.5±4.6	0.152

*****P value <0.05 is significant according to Mann Whitney U Test or student's t test

No significant difference was observed in levels of hs CRP, Apo A and Apo B100 levels in three groups.

3.3 Correlation of various cardiac biomarkers

When the correlation of various cardiac biomarkers with each other was seen, it was observed that sCD40L, Apo A and ApoB depicted no coorelation with any other parameter. Troponin T had a positive correlation with hs CRP (rho= 0.241*; p= 0.037) and total cholesterol(rho= 0.229*; p= 0.048).

3.4 ROC curve analysis of sCD40L, TroponinT and CRP in ACS

ROC curve analysis was done to assess the diagnostic efficiency of sCD40L, TroponinT and CRP to differentiate between less severe and more severe forms of the disease. It revealed that the sCD40L was better marker to differentiate between less and more severe forms of ACS with area under the curve (AUC) of 0.783 with p value of <0.001. At a cut off value of 1.22, sCD40L was found to be 82% sensitive and 62% specific. For Troponin T and CRP AUC was 0.551 (p value = 0.485) and 0.548 (p value = 0.518) respectively (figure 1).

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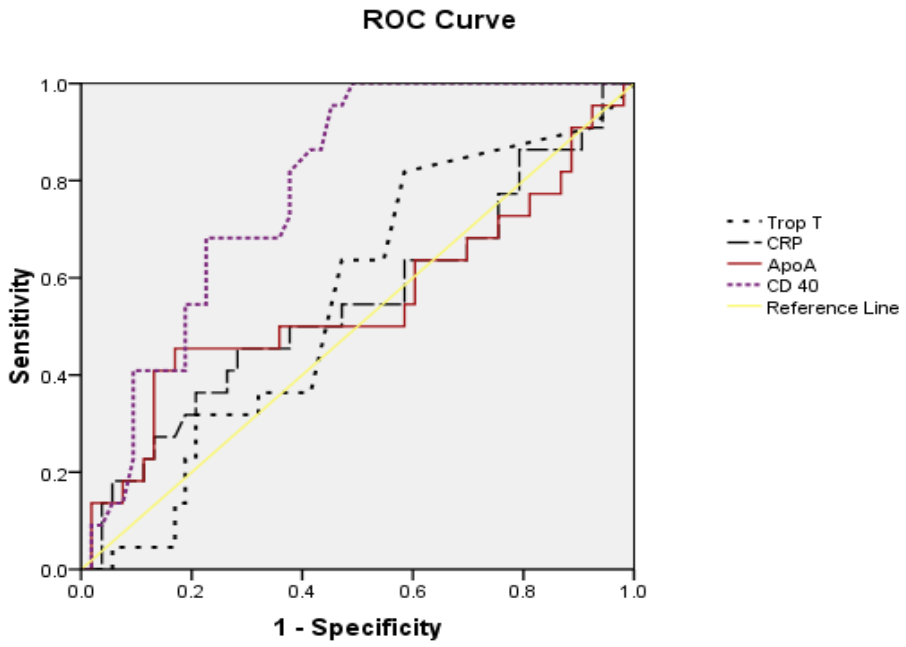

Figure 1 receiver operating characteristic (ROC) curve analysis to determine the diagnostic efficiency of sCD40L, hs CRP and hs Troponin T and Apo A.

4. DISCUSSION

Inflammation contributes to many of the characteristics of atherosclerotic plaque implicated in the pathogenesis of acute coronary syndromes, including rupture of the plaque's fibrous cap and a superficial erosion of the intima.[11] The fibrous cap rupture undeniably involve inflammation however superficial erosion has a less clear relationship with inflammation.

CD40, an integral membrane protein and its cognate agonist CD40 ligand, a 39-kD transmembrane protein structurally related to tumor necrosis factor-alpha, are co-expressed by several cells like monocyte/macrophages, endothelial cells, smooth muscle cells, and platelets.[5] CD40 ligand also occurs in a soluble form (sCD40L) that is considered to possess full biological activity. sCD40L is a proinflammatory marker that has been shown to be a promoter of atherosclerosis and plaque instability. Elevated levels of sCD40L have been found in those exhibiting hypercholesterolemia, unstable angina, or acute myocardial infarction. [3] It has strong independent prognostic value in healthy individuals and in patients with acute coronary syndrome (ACS).[7,8]

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However, the findings of studies of CD40L/sCD40L have been inconsistent. Some studies [12-14] have been performed to evaluate the relationship between coronary angiographic findings and serum sCD40L levels, these give results which are confounding.

Fouad et al[12] found no relation between serum levels of sCD40L and culprit lesion angiographic anatomy or number of vessels in patients with ACS., thus they concluded that sCD40L seemed to be a clinical marker of plaque instability rather than the extent of atherosclerosis.

Griva et al[13] measured several novel biomarkers in patients with stable CAD, including macrophage chemoattractant protein-1, matrix-metalloproteinase-3, soluble tumor necrosis factor receptor- 2 and sCD40L. The severity of Coronary Artery Disease (CAD) was graded with the SYNTAX score. The results suggested that in patients with stable CAD, sCD40L does not have any association between with the grade of CAD. But in a more recent study sCD40L levels were independently associated with angiographic severity of coronary artery disease in patients with ACS.[14] In a study looking for association between circulating level of CD40 ligand and angiographic morphologic features indicating high-burden thrombus formation in patients with acute myocardial infarction undergoing primary coronary intervention they found that the circulating level of sCD40L was the most independent predictor of the angiographic morphologic features of high-burden thrombus formation.

Our results show a significant relation of serum sCD40L levels with angiographic vessel score (p=0.000) which is mirrored by many recent studies. On the other hand, hs-CRP levels were insignificant. These findings point to progressing and worsening CAD being associated with high sCD40L levels. But the studies also suggest that this was probably due to our patient subgroup of only ACS, where sCD40L has been labelled as a tentative biomarker for correlation with angiographic and morphologic features but in CAD such a scenario is not seen.[15]

Our study revealed that CRP was raised in all three groups above the normal cut off range although showed no significant difference among single, double and triple vessel disease. CRP was related to Troponin T but no significant correlation was observed with sCD40L or lipid markers. On contrary, in a similar study it was observed that triple vessel disease patients had significantly higher CRP levels than one vessel and two vessel disease, while the difference was not significant between one and two vessel disease groups [12].

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CAD has been known to be positively correlated with apolipoprotein B-100 and inversely with apolipoprotein A1. In our study the mean values of lipid parameters viz. total cholesterol (TC), LDL-C and HDL-C and the lipid ratios were significantly different in the three groups, total cholesterol, LDL and HDL being higher in third group. Also mean levels of Apo A and Apo B did not show any correlation with angiographic vessel score although Apo B-100 was higher in group 1 with least angiographic blockage. This could be attributed to the hypolipidemic drug therapy (eg statins) being prescribed to the study subjects as most of the patients were taking these drugs at the time of intervention when their blood sample was drawn.

Study Limitations:

This was a pilot study which tried to correlate sCD40L with severity of ACS. Our study was mainly limited by being an observational study without comparison with healthy controls as they could not be subjected to coronary angiography. Also it did not include stable CAD patients but targeted only patients suffering from ACS. Moreover, it was a single centre study with small sample size of acute coronary syndrome.

Conclusion:

In conclusion, despite these limitations this study demonstrated that serum sCD40L levels were associated with angiographic severity in patients suffering from acute coronary syndrome. However, the cause of such a finding is due to accompanying inflammation found in the atherosclerotic lesions or due to some other cause cannot be determined. Additional studies are recommended to further illuminate the role of sCD40L in ACS.

Disclosure of interest: "No potential conflict of interest**."**

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