

INCREASED SERUM CONCENTRATIONS OF SOLUBLE CD40 LIGAND AS A PROGNOSTIC MARKER IN PATIENTS WITH ACUTE CORONARY SYNDROME

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ABSTRACT

CD40-CD40L interaction plays a significant role in the pathogenesis of atherosclerosis and coronary artery disease. The clinical predictive value of Soluble CD40 Ligand (sCD40L) was evaluated in patients with Acute Coronary Syndrome (ACS) and Non-Cardiac Chest Pain (NCCP). The levels of serum soluble CD 40 ligand were measured by ELISA in 485 patients admitted to emergency care unit, of which 89 patients were diagnosed as NCCP. The levels of sCD40L were significantly increased in patients with ACS when compared to controls and NCCP. Receiver Operator Characteristic (ROC) Curve analysis showed sCD40L to be a good discriminator between patients with ischemic heart disease and patients without ischemic heart disease. The area under the curve was found to be 0.940 with 95% CI (0.915 to 0.960) ($P < 0.0001$). The cut off value from the ROC curve was 2.99 ng/ml, above which sCD40L was considered to be positive. Combined assessment of sCD40L, Troponin I and CK-MB enhanced the risk prediction and early classification of patients. sCD40L seems to be a promising biomarker for identification and risk stratification for patients with acute coronary syndrome.

KEY WORDS

sCD40L, Atherosclerosis, Acute coronary syndrome, Biomarker.

INTRODUCTION

CD40, a trimeric transmembrane glycoprotein structurally related to TNF- α appears to play a crucial role in the pathogenesis of atherosclerosis and coronary artery disease (1, 2). Both membrane-bound and soluble forms of ligand interact with CD40, which is expressed in B-cells, macrophages, endothelial cells and vascular smooth muscle cells, platelets resulting in inflammatory responses. Upon platelet activation, CD40L is recruited onto the surface of platelet and cleaved into sCD40L which binds to CD40 receptor in the endothelial membrane dictating a flow of events

eventually leading to amplification of the inflammatory processes thus expelling increased sCD40L into the circulation. Soluble form of CD40L and CD40 acts as a mediator between platelets, vascular endothelium and other cell types (3). This biomarker should be focused on settings in which platelet activation is common, such as acute coronary syndromes or coronary revascularization procedures (4). Clinically relevant concentrations of human sCD40L increased the expression of its receptor CD40 in human coronary artery endothelial cells through a mechanism mediated by oxidative stress and extracellular signal-regulated kinase (ERK) 1/2 activation, suggesting a mechanism of amplification of CD40L biological function contributing to endothelial dysfunction and platelet activation in coronary endothelium (5). Elevated levels of sCD40L have been reported in patients with both stable or unstable angina and acute myocardial infarction. Heesch and his co workers established the predictive value of this marker by evaluating the clinical significance of sCD40L in patients presenting with chest pain (6). The present study evaluated the serum levels of sCD40L in patients with acute coronary syndrome and also analyzed the enhanced prediction of ACS on combined assessment of Troponin I, CK-MB and sCD40L.

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MATERIALS AND METHODS

Patients: The study was carried out at the Department of Biochemistry and Clinical Lab, International Centre for Cardio Thoracic and Vascular Diseases, Frontier Lifeline & Dr. K.M.Cherian Heart Foundation, Chennai, India. The study was approved by the Institutional Ethics Committee. The study group included 485 patients admitted to CCU with manifestations suggestive of acute myocardial ischemia, including those with chest pain with or without radiation, chest pressure, palpitations, shortness of breath, lower jaw pain, left arm pain, epigastric pain, hypotension, new or increasing lower extremity edema, syncope and other symptoms suggestive of angina. 12-lead ECG and all demographic details of the patients were recorded. Of the 485 patients admitted to CCU, 297 patients had acute coronary syndrome (98 patients with ST-segment elevation, 99 patients without ST segment elevation and 100 patients with unstable angina) with a mean age of 55±11 and 76% of them being males. Remaining patients were diagnosed as Non-Cardiac Chest Pain (NCCP) with a mean age of 52±11 and 67% being males. Among 99 healthy volunteers with no clinical evidence of heart disease had a mean age of 50±12 and proportion of males being 63%. Patients with liver and kidney disorders, brain ischemia and tumour were excluded from the study.

Blood Sampling: Venous blood was drawn from patients

admitted to CCU within 4-6 hours after symptom onset, into plain tubes (without anticoagulants) and allowed to clot for ½ an hour before centrifugation. Serum was separated and stored at -40°C until analysis and the samples were thawed only once.

Detection of sCD40L, Troponin I and CK-MB: Levels of sCD40L were determined using Bender Med systems (Austria) according to the manufacture’s instructions. The concentrations of troponin I and CK-MB were determined by MEIA (Abott AxSYM) and IFCC Method. Troponin I levels >0.1 ng/ml and CK-MB levels >25 U/L were considered positive

Statistical Analysis: Statistical evaluations were performed using SPSS software 9.0. ROC Curve analysis was performed using MedCalc 9.6. Data are expressed as Mean ± SD. Significance between subgroups were analysed using Krushkal Wallis test. P values <0.05 were considered statistically significant. Multivariate logistic regression was performed to analyze the significance of various parameters and risk factors involved in the study.

RESULTS

Patients’ history, biochemical parameters and risk factors for coronary artery disease were recorded (Table 1). From our study, sCD40L levels were significantly elevated in patients

Table 1: Baseline Characteristics of patients with acute coronary syndrome and control subjects

	Control	NCCP	STEMI	NSTEMI	UA	P Value
Biochemical Parameters (mg/dl)						
Glucose	108.34±23.93	142.18±47.77	214.54±91.88	170.27±75.03	184.23±81.34	0.001
Total Cholesterol	168.8±18.39	170.18±23.56	186.19±40.86	194.23±43.11	183.8±39.24	0.001
Triglycerides	148.14±33.20	148.95±45.87	157.67±63.20	140.82±44.33	157.49±67.10	NS
LDL	82.04±24.07	109.16±29.94	118.23±34.96	118.97±37.82	126.86±39.77	0.001
HDL	47.42±7.86	38.02±5.60	37.89±5.77	37.41±4.47	37.31±3.70	0.001
Creatinine	0.83±0.14	0.80±0.11	0.84±0.14	0.82±0.13	0.81±0.11	NS
Hemoglobin	14.09±1.58	13.66±1.60	14.16±1.55	13.66±1.54	13.55±1.52	NS
Risk Factors(%)						
Diabetes Mellitus	20	36	61	58	62	0.001
Hypertension	2	21	47	54	55	0.001
Family History of CAD	19	15	24	24	25	NS
Smoking	21	35	41	40	27	0.001
Alcoholism	11	21	19	21	10	0.001
Food Habits (Non-Vegetarian)	55	79	77	66	76	0.001

NCCP-Non-Cardiac Chest Pain; UA-Unstable Angina; NSTEMI-Non-ST Segment Elevation Myocardial Infarction; STEMI- ST Segment Elevation Myocardial Infarction

diagnosed with ACS (4.54 ± 1.73 ng/ml) when compared to controls (1.57 ± 0.83 ng/ml) (Fig 1) and NCCP (1.86 ± 1.06 ng/ml) (P Value < 0.001). ROC Curve analysis was done to demonstrate sCD40L levels as an individual risk determinant in patients with acute coronary syndrome. The optimum cut-off value above which sCD40L was considered positive is 2.99 ng/ml. The area under the curve was found to be 0.940 with 95% CI (0.915 to 0.960) (P Value < 0.0001). At the optimum cut off value 2.99 ng/ml, sensitivity and specificity were found to be 90% and 86% respectively. The levels of sCD40L in the subgroups of ACS were significantly elevated when compared to controls and NCCP (Fig 2).

DISCUSSION

The present study demonstrated that sCD40L may be a powerful biochemical marker of inflammatory thrombotic activity in patients with acute coronary syndrome. The increased levels of sCD40L reliably identify the sub-groups of



Fig 1: sCD40L levels in serum of control and ACS patients.

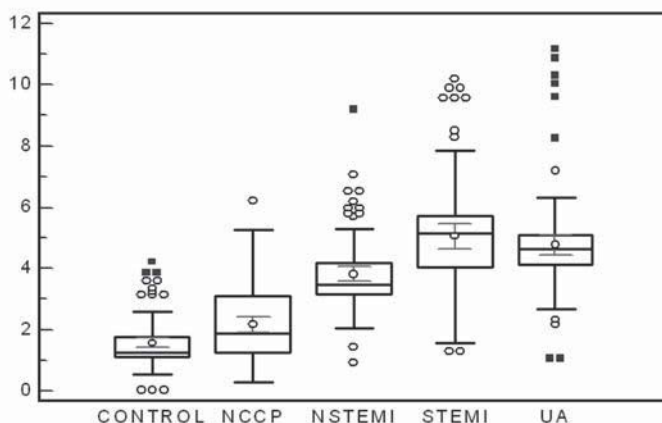


Fig 2: sCD40L levels in serum of control, NCCP and subgroups of ACS patients (UA, STEMI and NSTEMI)

patients with ACS. The sCD40L levels were higher non-significantly in patients diagnosed as unstable angina when compared to Non-ST Segment Elevation Myocardial Infarction (Table 2). As a matter of fact, sCD40L identifies the subgroup of patients at higher risk of cardiac events even among those with negative troponin. Positive troponin levels indicate myocardial necrosis whereas sCD40L levels reflect the inflammatory process of the culprit lesion. Since ACS is highly dependent on both the processes, an approach including measurement of both these markers may allow a better assessment of plaque instability (7).

Table 2: Serum levels of markers in subgroups of ACS on comparison with control and NCCP (Mean \pm SD)

Group	n	sCD40L (ng/ml)	Troponin I (ng/ml)	CK-MB (IU/L)
Control	99	1.57 ± 0.83	0.02 ± 0.04	19.47 ± 7.31
NCCP	89	1.86 ± 1.06	0.07 ± 0.42	20.17 ± 8.56
UA	100	$4.77^* \pm 1.64$	0.44 ± 2.23	29.63 ± 25.99
NSTEMI	99	$3.81^* \pm 1.19$	$2.34^* \pm 5.46$	$62.61^* \pm 74.53$
STEMI	98	$5.05^* \pm 2.02$	$6.65^* \pm 8.30$	$94.24^* \pm 87.84$

*P<0.0001—Control Vs Other Groups; †P<0.001— NCCP Vs Other Groups; NCCP-Non-Cardiac Chest Pain; UA-Unstable Angina; NSTEMI-Non-ST Segment Elevation Myocardial Infarction; STEMI- ST Segment Elevation Myocardial Infarction

Varo *et al* (8) reported that elevated levels of sCD40L identify patients with acute coronary syndrome at heightened risk of death and recurrent MI independent of other predictive variables, including cTnI and CRP. Aukrust *et al* (2) demonstrated that patients with unstable angina had significantly higher sCD40L compared with controls and stable angina. Recent studies suggest that sCD40L contributes to the progression of atherosclerotic and consequently to the destabilization of atherosclerotic plaques by inducing the expression of cytokines, chemokines, growth factors, matrix metalloproteinases and procoagulant factors in a variety of atheroma-associated cell types (9-11). Platelet activation is vital for the formation of thrombus which precipitates most unstable coronary syndromes. Previous studies have demonstrated that monocyte-platelet aggregates sensitively mark *in vivo* platelet activation than platelet surface P-selectin (12). Activated platelets produce and release larger amounts of sCD40L (9). Yan *et al* (13) have reported that serum soluble CD40L levels indicate an independent increased risk of major adverse cardiovascular events in patients with unstable coronary artery disease and also demonstrated correlation between platelet activation and sCD40L levels. Weber *et al* (14) and Ahn *et al* (15) have reported that the reliable sample

types for assessment of sCD40L were plasma when compared to serum. Tsuzuki et al (16) has observed no significant difference in serum sCD40L between UA and controls, in contrast to previously reported studies demonstrating increase in serum sCD40L in ACS patients. Ivandic et al (17) have reported significant increase of plasma sCD40L in ACS patients. However, in our study there was a significant increase in serum sCD40L levels in patients with ACS on comparison with controls.

Table 3: Sensitivity, Specificity, Area Under the Curve (AUC) and 95% CI of Troponin I, CK-MB and sCD40L for the diagnosis of Acute Coronary Syndrome

Parameter	AUC	95%CI	Sensitivity (%)	Specificity (%)
CK-MB	0.798	0.760 - 0.833	57	93
Troponin I	0.765	0.725 - 0.802	54	95
sCD40L	0.940	0.915 - 0.960	90	86

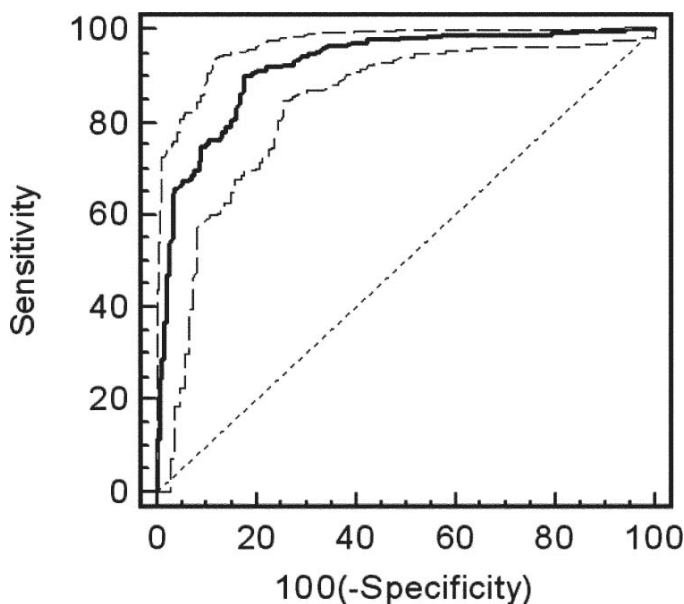


Fig 3: Receiver Operator Characteristic Curve (ROC) Analysis for the assay of sCD40L

ROC Curve analysis determines the optimum cut-off value above which sCD40L can be considered positive for the diagnosis of ACS. This study for the first time has depicted Receiver Operator Characteristic (ROC) curve for sCD40L in patients with acute coronary syndrome in Indian population. (Fig 3). Comparison of the area under the curve for Troponin I, CK-MB and sCD40L, reveals the fact that sCD40L may alone serve as a discriminator of patients with ACS. Combined analysis of sCD40L, Troponin I and CK-MB (Table 3, Fig 4) may enhance risk prediction for cardiovascular events. Initial estimation of sCD40L which signals inflammation, followed by serial testing of troponin can efficiently augment the diagnosis of acute coronary syndromes. No study demonstrating the increased sCD40L levels in serum for patients with ACS have been reported in Indian population. Multivariate logistic regression analysis revealed sCD40L levels to be highly significant on comparison with gold standards. Out of total study population (485), 297 subjects were diagnosed as ACS, of which sCD40L was positive in 267 samples on comparison with troponin (161) and CK-MB

Table 4: Multivariate Logistic regression of risk factors

Parameters	Coefficient	SE	95% Confidence Interval for Odds Ratio		Odds Ratio	P-value
Constant	-4.7681		-	-	-	-
Troponin	2.6890	0.6833	3.8250	55.7109	14.5978	0.0008*
CK-MB	1.3274	0.1633	1.3336	12.5475	3.7714	0.0304*
sCD40L	3.7979	0.4397	18.8399	105.6112	44.6061	0.0000*
Total Cholesterol	0.0192	0.0070	1.0054	1.0336	1.0194	0.0064*
Triglycerides	0.0013	0.0040	0.9934	1.0094	1.0014	0.7349
LDL	0.0148	0.0056	1.0037	1.0263	1.0149	0.0087*
HDL	-0.1224	0.0355	0.8523	0.9486	0.8848	0.0005*
Family history of CAD	0.4047	0.4713	0.5950	3.7753	1.4988	0.3906
Hypertension	1.0442	0.4353	1.2105	6.6691	2.8413	0.0164*
Smoking	0.7217	0.4716	0.8167	5.1859	2.0579	0.1259
Alcoholism	-0.6909	0.5471	0.1715	1.4643	0.5011	0.2066
Food habit	0.5603	0.4315	0.7518	4.0797	1.7513	0.1941

*P<0.05 significant at 5% level

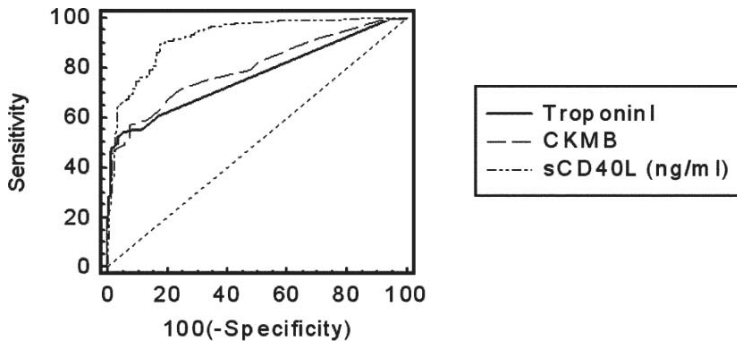


Fig 4: ROC Curve Analysis of sCD40L, Troponin I and CK-MB

(169). In contrast, false positives are still the undesirable outcomes. In this study population, Glucose was found to be significantly increased in patients with ACS and this denotes the onset of diabetes, a major risk factor for coronary artery disease. Hypertension, hypercholesterolemia, LDL cholesterol being major risk factors apart from diabetes were also found significant (Table 4). Our results were in agreement with Aukrust et al who demonstrated increased levels of sCD40L in ACS patients and also diagnosis of patients with ACS in emergency care unit.

In conclusion, sCD40L elevation may constitute a reliable marker of inflammation and independently stratify the patients with ACS. However, the restraint of this study is smaller sample size and further larger patient studies should be performed to illustrate the clinical use of sCD40L as an independent risk predictor and also importantly for triage of patients admitted to the coronary care unit.

REFERENCES

- Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, Kroczeck RA. CD40 Ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* 1998; 391: 591-4.
- Aukrust P, Muller F, Ueland Thor BS, Berget T, Aaser E, Brunsvig BS, *et al*. Enhanced levels of soluble and membrane bound CD40 Ligand in platelets with unstable angina. *Circulation* 1999; 100: 614-20.
- Mach F, Schonbeck U, Libby P. CD40 signaling in vascular cells: a key role in atherosclerosis? *Atherosclerosis* 1998; 137(suppl): s 89-95.
- de Lemos JA, Zirlik A, Schonbeck U, Varo N, Murphy SA, Kera A, *et al*. Associations between soluble CD40 Ligand, Atherosclerosis Risk Factors and Subclinical Atherosclerosis. Results from the Dallas Heart Study. *Arteriosclerosis, Thromb Vascular Biol* 2005; 25: 2192-6.
- Chai H, Yan S, Wang H, Zhang R, Lin PH, Yao Q, Chen C. CD 40 ligand increases expression of its receptor CD40 in human coronary artery endothelial cells. *Surgery* 2006; 140: 236-42.
- Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, Simoons ML; CAPTURE Study Investigators. Soluble CD40 Ligand in acute coronary syndromes. *N Engl J Med* 2003; 348: 1104-11.
- Santilli F, Basili S, Ferroni P, Davi G. CD40/CD40L system and vascular disease. *Inter Emerg Med* 2007; 2: 256-68.
- Varo N, de Lemos J A, Libby P, Morrow DA, Murphy SA, Nuzzo R, *et al*. Soluble CD40L risk prediction after acute coronary syndromes. *Circulation* 2003; 108: 1049-52.
- Henn V, Steinbach S, Buchner K, Presek P, Kroczeck RA. The inflammatory action of CD40 ligand expressed on activated human platelets is temporally limited by coexpressed CD40. *Blood* 2001; 98: 1047-54.
- Mach F, Schonbeck U, Bonnefoy JY, Pober JS, Libby P. Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: Induction of collagenase stromelysin and tissue factor. *Circulation* 1997; 96: 396-9.
- Miller DL, Yaron R, Yellin MJ. CD40L-CD40 interactions regulate endothelial cell surface tissue factor and thrombomodulin expression. *J Leukoc Biol* 1998; 63: 373-9.
- Michelson AD, Barnard MR, Krueger CA, Valeri CR, Furman MI. Circulating monocyte - platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-Selectin: Studies in baboons, human coronary intervention and human acute myocardial infarction. *Circulation* 2001; 104: 1533-37.
- Yan J-C, Zhu J, Gao L, Wu ZG, Kong XT, *et al*. The effect of elevated serum soluble CD40 ligand on the prognostic value in patients with acute coronary syndrome. *Clin Chim Acta* 2004; 343: 155-9.
- Weber M, Rabenau B, Stanisch M, Nef HM, Mollmann H, *et al*. Influence of sample type on soluble CD40 ligand assessment in patients with acute coronary syndromes. *Thromb Res* 2007; 120: 811-14.
- Ahn RE, Lander G, Jy W, Bidot JC, Jimenez JJ, Horstman LL, Ahn SY. Differences of soluble CD40L in sera and plasma: Implications of CD40L as a marker of thrombotic risk. *Thromb Res* 2004; 114: 143-8.
- Tsuzuki M, Morishima I, Yoshida T, Hayashi Y, Miura M, Hirai T, *et al*. Inverse correlation between soluble CD40 ligand and soluble CD40 is absent in patients with unstable angina. *Heart Vessels* 2005; 20: 245-50.
- Ivancic TB, Spanuth E, Hasse D, Lestin HG, Katus AH. Increased plasma concentrations of sCD40L in acute coronary syndrome depend on in vitro platelet activation. *Clin Chem* 2007; 53(7): 1231-4.