

## A study of Serum Soluble CD40 Ligand level and its correlation with serum CK-MB and lipid profile in patients with acute coronary syndrome

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### Abstract

**Introduction:** Coronary heart disease is an impairment of heart function due to inadequate blood flow to the heart. It is the most common cause of death worldwide. Serum sCD40L is an inflammatory marker. It is released from the platelets as soon as the onset of the chest pain. It is a strong predictor of cardio vascular risk factor.

**Objectives:** To estimate Serum sCD40Ligand, Serum CK-MB, Serum Lipid Parameters, Serum LDH, and Serum AST levels in patients with ACS.

**Materials and Methods:** Study group comprised of 50 subjects were admitted in ICCU with ACS. 50 healthy sex and age matched subjects were taken as control groups. Venous blood samples were collected. Serum Soluble CD40Ligand was estimated by ELISA technique, Serum CK-MB estimated by Immunoinhibition method, Serum Lipid profile by enzymatic assay methods. Serum LDH by DKG method, Serum AST by Modified IFCC method.

**Results:** There was significant increase in Serum sCD40L ( $p < 0.0001$ ), Serum TC, Serum TGL, Serum LDL-C, Serum VLDL while significant decrease in the Sr. HDL-C levels in the ACS patients compared to control group. There was no significant change in Sr. LDH levels and Sr. AST levels in ACS patients compared to control group. There was a significant positive correlation between Serum sCD40L and Serum CK-MB, Serum TC, Serum TGL, Serum LDL-C, Serum VLDL and a negative correlation between Serum sCD40L and Serum HDL-C while no significant correlation was found between Serum sCD40L with Serum LDH and Serum AST levels.

**Conclusion:** Serum sCD40L can be used as a marker in individuals with Acute Coronary Artery disease.

**Keywords:** sCD40L, ACS, MI, CK-MB, Lipid Parameters.

### Introduction

Coronary heart disease is defined as cardiac function impairment. There is an inadequate blood flow in the heart compared to its needs and obstructive changes in the coronary circulation.<sup>1</sup> CHD is a worldwide health epidemic. All over the world it is the commonest cause of death. In USA, European countries, Japan, Singapore, Canada etc, this is the first common cause of death. In India it is one in ten common causes of death.<sup>2</sup> Epidemics of CHD in United States, began in the early 1920s, in the 1930s in the Britain, still later in several European countries. Worldwide cardiovascular death is about 30 percent. Of which CHD is more than half.<sup>3</sup> CHD has been classified as Acute Coronary Syndrome, Chronic CHD, and sudden death. Clinically in many ways CHD may present, extending from an asymptomatic finding to unexpected cardiac collapse. Chronic CHD is always secondary to coronary atherosclerosis, leading to coronary blood flow mismatch and adenosine triphosphate homeostasis (imbalance of demand and supply) and coronary ischemia.

Acute coronary syndrome is a unifying term representing a common end result, acute myocardial ischemia. Acute ischemia is usually caused by

atherosclerotic plaque rupture, erosion, fissuring, or a combination with superimposed intracoronary thrombosis and is associated with an increased risk of myonecrosis and cardiac death. It encompasses acute myocardial infarction and unstable angina (resulting in ST elevation or non-ST elevation).

ACS in emergency department should be triaged immediately to an area with defibrillation capability and continuous electrocardiographic monitoring.<sup>4</sup> Diagnosis of ACS at this earlier stage can prevent morbidity and mortality. Along with ECG changes, proteins in the serum like CK-MB, Cardiac Troponin-I & T, LDH and AST are used to diagnose ACS.<sup>5</sup> However, in the absence of necrosis these markers are not elevated when measured in the first 2-6 hours following an ischemic event.

Serum sCD40 Ligand - is a type II transmembrane glycoprotein. It is structurally related to Tumor Necrosis Factor -alpha. It is expressed within seconds after platelet activation and it has been shown to be pro-inflammatory for endothelial cells, which ultimately lead into endothelial dysfunction and atherosclerosis.<sup>6</sup> Hence in the present study, serum levels of sCD40L were estimated in patients with ACS and the relationship between Serum sCD40L, Serum CKMB and lipid profile were analyzed

### Aims and objectives

1. To estimate Serum sCD40L level in patients with Acute Coronary Syndrome.
2. To correlate the levels of Serum sCD40L with Serum CK-MB which is an effective marker of Acute Coronary Syndrome.
3. To correlate the levels of Serum sCD40L with Lipid profile.

### Materials and Methods

The study was conducted at Thanjavur Medical College Hospital after getting the approval from the ethical committee. In the present study the age group of both study and control group ranged from 35-65 years, males and females were included and informed consent obtained from them. 50 subjects (30 males and 20 females) who were admitted in ICCU of Thanjavur Medical College Hospital with Acute Coronary Syndrome and with clinical findings suggestive of STEMI (n=33), NSTEMI (n=4) and unstable angina (n=13) were included in the study group. 50 sex and age matched, healthy individuals were taken as control groups.

#### Inclusion criteria

1. Patients admitted with complaint of chest pain within 6 hours of onset, chest pain lasting >30 minutes.
2. Electrocardiographic findings showing abnormal ST-T wave changes (ST segment elevation > 1mm in two or more contiguous chest leads or depression or deep symmetrical T wave inversion).
3. Elevation of serum CK-MB levels more than the normal range.

#### Exclusion criteria

1. Anaemia
2. Infection
3. Malignant disease
4. Collagen disease
5. Stroke
6. Cardiac disease other than coronary disease
7. Ballon angioplasty
8. Overt right or left ventricular failure
9. Thrombolytic treatment within three months of study

#### Blood collection

Blood samples were collected by venipuncture with strict aseptic precaution as soon as the subjects got admitted as per the inclusion criteria. All the blood samples were centrifuged at 3000 rpm for 10 minutes

and serum separated. One part of the serum sample was taken for analysis of CK-MB, Creatinine, and Urea. The remaining part of the serum sample was stored for analysis of soluble CD40L at -40°C 12-14 hours. Fasting blood sample was also collected from all subjects during their hospital stay and analysis of total cholesterol, TGL, HDL-C, fasting blood sugar were done.

**Estimated parameters from blood samples:** The serum sample collected above was used for the estimation of the biochemical parameters. Serum sCD40L was estimated by Enzyme Linked Immuno Sorbant Assay based on the sandwich principle, according to the manufacturer's instructions.<sup>7</sup> Serum CK-MB was estimated by Kinetic immuno inhibition method.<sup>8</sup> FBG was measured by Trinders method.<sup>9</sup> Blood Urea was measured according to Urease – GLDH method.<sup>10</sup> Serum Creatinine was measured according to Jaffes method.<sup>11</sup>

The estimation of Total cholesterol was done by Cholesterol oxidase-PAP,<sup>12</sup> Serum Triglycerides by GPO-PAP method,<sup>13</sup> HDL-C by Phosphotungstic acid method<sup>14</sup> and LDL-C and VLDL-C was calculated as described by Friedewald et al.<sup>15</sup> Serum LDH was estimated by modified IFCC Method<sup>16</sup> and Serum AST was by DGKC Method.<sup>17</sup>

#### Statistical analysis

Student's 't' test and Chi-square test were employed for the statistical analysis of data. The data were expressed in terms of mean and standard deviation. P value less than 0.05 was taken as significant value. Correlation between the measured parameters was assessed using Pearson's coefficient of correlation.

### Results

#### Baseline parameters in patients with acute coronary syndrome and control group

**Age:** The mean age in study group was 55.56 ± 9.53 yrs and control group was 52.22 ± 9.41 yrs. The mean weight in study group was 66.84 ± 7.75 kg and control group was 66.60 ± 7.19 kg. The mean height in study group was 1.63 ± 0.07 m and control group was 1.61 ± 0.07 m. The mean BMI in study group was 25.29 ± 2.83 and control group was 25.35 ± 1.72. There was no significant difference in age, weight, height and BMI of study group and control group shows the control group taken was age and sex matched. Similarly there was no significant difference in Blood pressure, blood urea levels and Serum creatinine levels in both Study and control group. (Table 1)

**Table 1: Baseline characteristics of Study population**

S.No.	Variables	Control (n=50)	Study (n=50)
1	Age (yr)	52.22 ± 9.41	55.56 ± 9.53
2	Wt(Kg)	66.60 ± 7.19	66.84 ± 7.75
3	Ht (m)	1.61 ± 0.07	1.63 ± 0.07

4	BMI	25.35 ± 1.72	25.29 ± 2.83
5	SBP (mm Hg)	117.62 ± 8.00	118.72 ± 22.18
6	DBP (mmHg)	78.08 ± 4.462	82.00 ± 11.56
7	FBG (mg/dl)	93.94 ± 6.97	110.12 ± 35.34
8	B. Urea (mg/dl)	25.82 ± 4.60	25.36 ± 4.65
9	Sr.Creat(mg/dl)	0.68 ± 0.12	0.7 ± 0.084

**Serum soluble CD40 ligand levels, Serum soluble CK-MB levels, Lipid profile and Liver function parameters between patients with acute coronary syndrome and control**

Mean serum sCD40L level in ACS patients group was (4.397 ng/ml) significantly higher than the control group (1.422 ng/ml (P=0.0001<0.05). There was significant increase in mean Sr. CK-MB level in ACS group (56.08± 22.58 U/L) compared to control group (13.48±4.047 U/L) (P <0.05). There was a statistically significant elevation of Sr.TC, Sr. TGL, Sr. VLDL-C, Sr. LDL-C, in ACS group compared to control (P=0.0001<0.05). The Sr.TC level was 262.12 ± 39.72 mg/dl in ACS patients compared to 166.98 ± 17.75 mg/dl in control group. The serum triglycerides level was 185.04 ± 43.41 in ACS patients compared to 124.76 ± 23.67 mg/dl in control group. The serum Sr. LDL-C and VLDL-C levels were 191.16 ± 40.13 mg/dl and 36.80 ± 8.75 mg/dl in ACS patients respectively compared to 98.90 ± 21.36 mg/dl and 24.86 ± 4.75 mg/dl respectively in control group. There was statistically significant decrease in the Sr. HDL-C levels in the study group compared to control group. The serum Sr. HDL-C level was 34.16 ± 3.88 mg/dl in ACS patients compared to 43.24 ± 9.90 mg/dl in control group. There was no significant change in Sr. LDH levels and Sr. AST levels in ACS patients compared to control group. (Table 2)

**Table 2: showing comparison of Lipid profile parameters between patients with acute coronary syndrome and control group**

S. No	Variables	Control (n=50)	ACS patients (n=50)
1	Sr.sCD40L (ng/ml)	1.42 ± 0.139	4.39 ± 1.90*
2	Sr.CK-MB (U/L)	13.48 ± 4.04	56.08 ± 22.58*
3	Sr.TC (mg/dl)	166.98 ± 17.75	262.12 ± 39.72*
4	Sr.TGL (mg/dl)	124.76 ± 23.67	185.04 ± 43.41*
5	Sr.HDL-C (mg/dl)	43.24 ± 9.90	34.16 ± 3.88*
6	Sr.LDL-C (mg/dl)	98.90 ± 21.36	191.16 ± 40.13*
7	Sr.VLDL-C (mg/dl)	24.86 ± 4.75	36.80 ± 8.75*
8.	Sr.LDH (U/L)	139.00 ± 11.32	148.86 ± 22.37
9.	Sr. AST (U/L)	15.02 ± 2.93	17.04 ± 4.62

**Pearson’s correlation between Serum soluble CD40 ligand levels and biochemical parameters in patients of acute coronary syndrome**

There was a significant positive correlation between Serum sCD40L and Serum CK-MB, Serum TC, Serum TGL, Serum LDL-C, Serum VLDL and a negative correlation between Serum sCD40L and Serum HDL-C which is statistically significant. No significant correlation was found between Serum sCD40L with Serum LDH and Serum AST. Increased level of sCD40L by 1 ng/ml leads to increase in level of Sr.CK-MB by 0.777 U/L, Sr.TC by 0.693mg/dl, Sr. TGL level by 0.596mg/dl, Sr. LDL-C level by 0.586mg/dl and Sr. VLDL-C 0.620mg/dl respectively in ACS patients. Increased level of sCD40L by 1 ng/ml leads to decrease in level of Sr. HDL-C level by 0.637mg/dl. (Table 3)

**Table 3: Showing Pearson’s correlation between Serum soluble CD40 ligand levels and biochemical parameters in patients of acute coronary syndrome**

S. No	Variables	Pearson Correlation coefficient Value
1	Sr.sCD40L (ng/ml)	1
2	Sr.CK-MB (U/L)	0.777**
1	Sr.TC (mg/dl)	0.693**
2	Sr.TGL (mg/dl)	0.596**
3	Sr.HDL-C (mg/dl)	- 0.637**
4	Sr.LDL-C (mg/dl)	0.586**
5	Sr.VLDL-C (mg/dl)	0.620**
6	Sr.LDH U/L	-0.082
7	Sr.AST U/L	-0.031

**Discussion**

The present study was done to evaluate levels of Serum sCD40L in patients with Acute Coronary Syndrome. It is a powerful biochemical inflammatory

marker. In our study mean age of study group was (55.56± 9.53 years) and that of control group was (52.22± 9.41 years), mean BMI of study group and control group were equal and there was no statistical significance (25.29 ± 2.83 versus 25.35 ± 1.72, P>0.05).

In the present study mean serum sCD40L level of study group was significant higher than the control group (4.39 ± 1.90ng/ml versus 1.42 ± 0.139 ng/ml, P<0.05). This findings are in accordance with the study of Valerio Sanguigni et al, (4.18 ± 2.07 ng/ml in study group versus 2.60 ± 0.7 ng/ml in control groups).<sup>18</sup> Also our study finding was almost consistent with Pal Aukurst et al study and Priya Gururajan et al study which reported higher levels of Serum sCD40L in ACS patients.

The mean Serum total cholesterol level of 262.12 ± 39.72mg/dl in the study group was significantly higher than the control mean Serum cholesterol level of 166.98 ± 17.75 mg/dl. This level was slightly higher than the mean level of Serum cholesterol observed in a study done by Priya Gururajan et al, and Mari Luomala et al.<sup>19</sup>

The mean Serum TGL level was 185.04 ± 43.41mg/dl which is significantly higher than the control group mean of 124.76 ± 23.67mg/dl. Serum TGL level increases from 90 mg/dl to 180 mg/dl is associated with the doubled the incidence of CAD.<sup>20-22</sup>

The mean Serum HDL cholesterol level of 34.16 ± 3.88mg/dl in the study group was significantly lower than the control group mean Serum HDL cholesterol level of 43.24 ± 9.90mg/dl. Similar values were observed in Yogendrasingh et al, study and demonstrated that low Serum HDL-C increases the risk of CAD.

The mean Serum LDL-C level of 191.16 ± 40.13mg/dl in the study group was significantly higher than the control group mean Serum LDL-C level of 98.90 ± 21.36mg/dl. Raised Serum LDL cholesterol has been recognized as a primary risk factor for CAD.<sup>22</sup>

The mean level of Serum CK-MB value in the study group was higher than the mean level of Serum CK-MB in control groups (56.08±22.58 U/L versus 13.48±4.04 U/L), which is statistically significant (p<0.05).

These variations can be attributed to the modifiable and non-modifiable risk factors of atherosclerosis. Serum LDH and Serum AST values were not that much raised in all age groups.

Pearson coefficient correlation analysis in the study group shows there is a highly significant positive correlation between Serum sCD40L and Sr.CK-MB (r=0.777, p<0.01) Sr.TC(r=0.693, p<0.01) Sr.TGL(r=0.569, p<0.01) There is negative correlation between Serum sCD40L and Serum HDL-C which was statistically significant (r=-0.637 p<0.01).<sup>23,24</sup>

Serum sCD40L contributes to atherosclerotic plaque destabilization and progression of chemokines,

growth factors, cytokines and procoagulant factors in various cell types associated with atheroma. For thrombus formation, platelet activation is important, which in turn leads to precipitation of most of the unstable coronary syndromes. Michelson AD et al found that large amounts of Serum sCD40L are produced and released from activated platelets. Yan et al in their study, demonstrated correlation between sCD40L and platelet activation. They also found that in patients with CAD, Serum sCD40L levels indicate an independent increased risk of major adverse cardiovascular events.

## Conclusion

Biochemical markers such as Serum CK-MB, cardiac Troponin-I, and Myoglobin are used in the clinical setting to assess MI. However, elevation of these markers indicate myocardial necrosis and in the absence of necrosis these markers are not elevated. Serum sCD40L is a marker of inflammatory thrombotic activity that is expressed within seconds, after platelet activation. Platelet activation and elevated fibrinogen level are associated with increased risk of Coronary thrombosis which is the gravest complication of atherosclerosis. Since ACS is complicated by both myocardial necrosis and inflammation, assessment of both the processes may allow a better assessment of the disease. Early intervention using anti-inflammatory drugs can be tried to prevent the progression of infarct size. This helps in reducing the morbidity and mortality from acute coronary syndrome.

## Limitations of the study

Serum sCD40L when coupled with coronary angiography would have aided in assessing the severity of coronary stenosis. Analysis of genetic polymorphism in sCD40L would have enabled discrimination of various iso forms and their association with CAD

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