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RESEARCH ARTICLE

The Role of Serum sCD40L in Acute Coronary Syndrome.

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Abstract

Background: Acute coronary syndrome (ACS) is caused by the sudden formation of blood clots within a coronary artery. The blood clots are caused by rupture of a plaque in the wall of the artery. Platelet aggregation is a complex reaction involving multiple mechanisms in platelet activation; it plays a significant role in the etiology of cardiovascular disease. Serum sCD40 ligand (sCD40L) is a biomarker of platelet activation. The aim of this study was to highlight the role of serum sCD40L in ACS patients as platelet activation marker for early detection and prevention of acute myocardial infraction (AMI). Patients and methods: The study involved 40 subjects with coronary affection (15 patients with unstable angina (UA) and 15 subjects with acute MI and 10 patients with depressed ST segment with MI) and 40 apparently healthy subjects as control group. Patients and control were subjected to carful history taking full clinical examination, ECG, echocardiography, lipid profile, creatine kinase (total and MB), troponin-I and serum sCD40L. Results: There was highly significant increase of serum sCD40L in all ACS patient groups when compared to control group. There was marked increase in serum sCD40L in patients with AMI compared with other patient groups. There was significant positive correlation between serum sCD40L and serum creatine kinase (CK) (total & MB), serum total cholesterol, serum triglyceride and serum LDL and significant negative correlation with serum HDL. Conclusion: Serum sCD40L can be used for early detection of platelet activation in ACS to avoid the risk of acute MI complications. We recommend its use as a marker of platelet activation in ACS.

Introduction

Acute coronary syndrome refers to a spectrum of clinical presentations ranging from those for ST-segment elevation myocardial infarction (STEMI) to presentations found in non–ST-segment elevation myocardial infarction (NSTEMI) or in unstable angina (UA). In terms of pathology, ACS is almost always associated with rupture of an atherosclerotic plaque and partial or complete thrombosis of the infarct-related artery **(1).**

In some instances, however, stable coronary artery disease (CAD) may result in ACS in the absence of plaque rupture and thrombosis, when physiologic stress (eg, trauma, blood loss, anemia, infection, and tachyarrhythmia) increases demands on the heart. The diagnosis of acute myocardial infarction in this setting requires a finding of the typical rise and fall of biochemical markers of myocardial necrosis in addition to at least 1 of the followings: ischemic symptoms, development of pathologic Q waves and ischemic ST-segment changes on electrocardiogram**(1)**.

Myocardial infarction (MI) is considered part of a spectrum referred to ACS. The ACS represents ongoing myocardial ischemia or injury consists of unstable angina, non-ST segment elevation of myocardial infarction (NSTEMI) and ST segment elevation of myocardial infarction (STEMI) (**2).**

Inappropriate platelet activation can lead to arterial thrombosis, the formation of a blood clot within a blood vessel to trigger heart attack and MI **(3).** CD40L is a potent platelet derived cytokine; it is α protein of platelet granules which is also rapidly presented at the platelet surface after platelet stimulation. The surface expressed CD40L is subsequently cleaved from the cell over a period of minutes to hours, from where it diffuses into the plasma as a soluble trimeric fragment termed sCD40L. It was found that more than 95 % of circulating sCD40L originates from platelets **(4).** Proinflammatory and prothrombotic mediators including CD40L are biomarkers of platelet activation **(5).**

We aimed to highlight the role of serum sCD40L in ACS patients as platelet activation marker for early detection and prevention of acute MI.

Patients and methods:

The study was conducted from March 2013 to March 2014 on 40 patients with acute attack of typical chest pain, chosen from those attending emergency unite in Al-Zahra University Hospital and were admitted in cardiac care unit and in ICU. Approval of the ethical committee of faculty of medicine, AL-Azhar University on the study was obtained. An informed written consent from each subject participating in the study was also obtained. From these patients 15 suffered from acute ST segment elevation MI, 15 subjects from unstable angina and 10 patients from ST segment depression MI. They were 18 females (45 %) and 22 males (55%) with an age ranged 44 and 73 years with mean of 57.30±8.33years. The study also included 40 apparently healthy subjects as a control group. They were 19 females (47.5%) and 21 males (52.5%) with age ranged 35 and 70 years with mean of 60.08±6.85 years.

The studied subject were divided into four groups
Patients with AMI (Group A): **Fifteen patients showed typical signs of acute myocardial infarction.**
The inclusion criteria were:

- **Typical angina pain lasting > 30 minutes**
- **ST segment elevation of > 1 mm in two or more contiguous leads**
- **Elevation of serum CK to > 3 times the upper limit of normal with a concomitant rise in the CK- MB isoenzyme (CK-MB).**

Patients with ST segment depression MI (Group B): **Ten patients fulfilling the criteria for ST segment depression MI. There was evidence of minor myocardial injury according to their electrocardiograms, which revealed transient ST segment depression and T wave inversion, and from troponin I measurement results.**

Patients with UA (Group C): **Fifteen patients fulfilling the criteria for unstable angina, they had no evidence of "major" myocardial necrosis, as reflected by raised serum CK or CK MB isoenzyme activity.**

Control group (Group D): **Forty healthy volunteers, age and sex matched.**

Exclusion criteria**: Patients with chronic renal failure and liver cell failure**

Patients and control groups were subjected to:

- **Carful history taking and full clinical examination.**
- **Electrocardiography (ECG) and echocardiography.**
- **Laboratory investigations included:**
  - **Complete blood count (CBC)**
  - **Lipid profile: total cholesterol and (LDL and HDL cholesterol), triglycerides, and CK (total and MB).**
  - **Serum troponin-I.**
  - **Serum sCD40L.**

Three ml of fasting (12-14 hours) venous blood samples were drawn from each subject participating in the study and left to clot then centrifuged at 1000 xg for 15 minutes and the serum was stored at -20°C for determination of laboratory parameters.

The determination of serum total cholesterol and serum triglyceride was done on Hitachi auto analyzer 736 (Hitachi, Japan). For determination of serum HDL, phosphotungestic acid and magnesium ions were used for precipitation of all lipoproteins except the HDL fraction which was present in the supernatant and measured by auto analyzer. LDL was calculated according to Friedwald formula (6)

Serum total CK was determined by kinetic UV method (7) **supplied from Intermediacy (Interemedical sri, Vallaricca, Italy). Serum CK-MB was measured by immunochemi-luminometric assay using kits supplied by Chemilumi ACS, Centaur, Bayer medical co Ltd, Tokyo, Japan as described by** (8).**Serum troponin I was determined qualitatively by rapid chromatographic immunoassay (**9**).**

Determination of serum sCD40L was performed using quantitative sandwich enzyme immunoassay technique (10**) and the kit was supplied from R&D Systems, Inc. (614 McKinley Place NE (612), Minneapolis, MN 55413, USA).**

Statistical analysis: Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples in comparing 2 groups when normally distributed. Comparison of numerical variables between more than two groups was done using one way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons. For comparing categorical data, Chi square (x) test was performed. Exact test was used instead when the expected frequency is less than 5. $P < 0.05$ was considered statistically significant. All statistical calculations were done using computer programs SPSS version 15 for Microsoft Windows.

Results:

The study was conducted on 40 patients with acute attack of typical chest pain, and divided into 3 groups: Group A included 15 patients with STEMI, group B included 10 patients with NSTEMI and group C included 15 patients with UA. Group D included 40 apparently healthy subjects as a control group. Serum troponin I was performed for all patients and was positive in 22 patients (55%) and negative in 18 patients (45%).

Table (1): General, clinical data and serum troponin of all studied patients.

Groups / Items	Group A N=15	Group B N=10	Group C N=15
Age(years) (mean±SD)	60.45±7.99	60.45±7.99	55.43±8.22
Gender			
• Male	11 (73.3%)	3 (30%)	8 (53.3%)
• Female	4 (26.6%)	7 (70%)	7(46.7%)
History of previous attack			
• Positive	10 (66.6%)	7 (70%)	10 (66.6%)
• Negative	5 (33.3%)	3 (30%)	5(33.3%)
Chest pain			
• Typical	15 (100%)	10 (100%)	15 (100%)
• Atypical	-	-	-

Group A: STEMI. Group B: NSTEMI. Group C: UA.

Table (2): Laboratory characteristics of the studied groups (mean ± SD).

Groups / Parameters	Group (A)	Group (B)	Group (c)	Group (D)	P
Cholesterol (mg/dl)	259.80±71.47	238.73±45.38	207.43±47.53	122.79±28.36	<0.05
Triglyceride (mg/dl)	183.40 ±48.42	173.27±47.33	136.36±50.95	102.05±24.31	<0.05
LDL (mg/dl)	95.53±28.72	99.91±26.86	82.93±21.83	85.50±14.96	<0.05
HDL (mg/dl)	30.53±6.96	39.70±9.10	37.71±6.03	44.88±11.76	<0.05
CK-total (IU/l)	822.07±547.31	276.27±214.29	100.93±43.65*	98.03± 31.89	<0.05
CK-MB (µg/l)	125.60± 142.50	72.82± 48.41	25.93± 10.69*	17.33± 3.31	<0.05

* Non significant elevation in serum CK (total, MB) in UA patient group when compared with control group.

Table (3): Serum sCD40L concentration in all studied groups (mean ± SD).

Groups / Parameter	Group A	Group B	Group C	Group D	P
sCD40L(ng/ml)	15.53±4.03	10.73± 2.45	7.46±1.51	2.91±1.06	<0.001

Table (4): Correlations between serum sCD40L and laboratory parameters in different studied groups.

	Serum sCD40L					
	Group A		Group B		Group C	
	R	P	r	P	r	P
CK total	0.661	<0.05	0.775	<0.05	-	-
CK-MB	0.513	<0.05	0.823	<0.05	-	-
Cholesterol	0.506	<0.05	0.817	<0.05	0.639	<0.05
Triglyceride	0.676	<0.05	0.518	<0.05	0.655	<0.05
LDL	0.573	<0.05	0.737	<0.05	0.573	<0.05
HDL	-0.212	<0.05	-0.122	<0.05	-0.111	<0.05

Table 1 showed general and clinical data of ACS patients:

Group A: 15 patients had STEMI. They were 11 males (73.3%) and 4 females (26.6%), with 10 cases with positive history of previous attack, (66.6%), and 5 with negative history (33.3 %), their chest pain was typical in all cases (100%), and their ECG with STEMI (100%).

Group B: 10 patients had NSTEMI. They were 3 males (30%) and 7 females (70%), with 7 cases with positive history of previous attack (70%) and 3 cases with negative history (30%), their chest pain was typical in all cases (100%), their ECG with NSTEMI (100%).
Group C: 15 patients had UA. They were 8 males (53.3%) and 7 females (46.7%), with 10 cases with positive history of previous attack (66.6%) and 5 cases with negative history (33.4%), their chest pain was typical in all cases (100%), their ECG with NSTEMI in 9 cases (60%) and 6 cases (40%) with no ECG change.

Table 2 revealed different laboratory characteristics of the studied groups. There was significant elevations in serum total cholesterol ($P<0.05$), serum triglyceride ($P<0.05$), serum LDL (in groups A and B only) ($P<0.05$), serum CK (total & MB) ($P<0.05$), and significant decrease in serum HDL ($P<0.05$) in all patients groups compared with control group. There was non significant elevation in serum CK (total, MB) in UA patient group when compared with control group.

Table 3 showed the concentration of serum sCD40L in different studied groups. There was highly significant elevation in serum sCD40L in all patient groups compared with control group ($P<0.001$). The elevation was marked in group A then group B then group C patients.

Table 4 demonstrated the correlation between serum sCD40L and different laboratory parameters in ACS patients. There was significant positive correlation between serum sCD40L and serum CK (total and MB), serum total cholesterol, serum triglyceride and serum LDL in patient groups A and B. There was significant positive correlation between serum sCD40L and serum total cholesterol, serum triglyceride and serum LDL in patient group C. Also there was significant negative correlation between sCD40L and serum HDL in all patient groups.

Discussion:

Acute coronary syndrome (ACS) refers to a spectrum of clinical presentations ranging from those for STSTEMI to presentations found in NSTEMI or in UA. ACS is almost always associated with rupture of an atherosclerotic plaque and partial or complete thrombosis of the infarct-related artery **(1).**

In some instances, however, stable coronary artery disease (CAD) may result in ACS in the absence of plaque rupture and thrombosis, when physiologic stress (eg, trauma, blood loss, anemia, infection, and tachyarrhythmia) increases demands on the heart. The diagnosis of acute myocardial infarction in this setting requires a finding of the typical rise and fall of biochemical markers of myocardial necrosis in addition to at least 1 of the following:

- Ischemic symptoms
- Development of pathologic Q waves
- Ischemic ST-segment changes on electrocardiogram **(1).**

The binding of CD40L to CD40 stimulates inflammatory processes including the release of proinflammatory cytokines and the expression of adhesion molecules implying in atherosclerosis. Patients exhibiting hypercholesterolemia, UA, or acute MI present with increased CD40L levels (**11).**

The aim of the study was to highlight the role of serum sCD40L in ACS patients as platelet activation marker for early detection and prevention of acute MI.

The study was conducted on 40 cases with ACS (STEMI (group A), NSTEMI (group B) and US (group C). The study also included 40 apparently healthy subjects as a control group (group D).

We found that a significant increase in serum total cholesterol, serum triglycerides and serum LDL in AMI and UA groups when compared to control group. These results were in agreement with **(12)** who demonstrated significant increase in serum triglyceride in ACS. Clinical evidence indicates that hypercholesterolemia and inflammation are essentially involved in the pathogenesis of CAD **(13).**

(14) described that retention and partial oxidation of LDL molecules within the intimae of the arterial wall creates a proinflammatory environment where the expression of endothelial adhesion molecules leads to the recruitment of monocytes from the circulation.

Creatine kinase and CK-MB represent the “gold standard” for the diagnosis of MI as defined by the World Health Organization criteria. CK is an enzyme present in many tissues, including the myocardium and skeletal

muscle. CK-MB is present in a relatively high concentration in the myocardium (roughly 20% of the total myocardial CK) **(15**).

Our study showed that a non significant increase of serum CK and CK-MB in UA group when compared to control group but significant increase of CK and CK-MB in AMI patients compared to control group. These results are in agreement with (**16),** who stated an elevation of CK & Ck-MB in AMI cases, which can be explained as necrosis of the cardiac muscle increasing CK & CK-MB, were found in AMI cases but not UA cases. The cardiac biomarkers CK and CK-MB play a critical role for the diagnosis of ACS, but they have a significantly delayed sensitivity. Even in the setting of cardiac necrosis 4–6 hours are required, before abnormal levels are measurable.

The present study revealed significant positive correlation between serum sCD40L and serum CK-MB in AMI which in agreement with (**17)**, who stated a positive correlation between sCD40L and extent of myocardial infarction, CK-MB.

The present study detected highly significant increase in serum sCD40L in all ACS patients group compared to controls. These results were in agreement with **(16) and** (18)**,** who reported expressions of CD40L on platelet and serum sCD40L were significantly higher in patients with ACS including UA and AMI than those in controls.

Increasing evidence suggests that blood platelets may be involved in the initiation of atheroma, modulate various inflammatory responses, and contribute to endothelial dysfunction and matrix-degrading processes of coronary atherosclerosis, in addition to their role in thrombosis. This has led to the hypothesis that platelet CD40 and platelet-derived sCD40 play pivotal roles in atherosclerosis, linking inflammatory and thrombotic components of the disease **(19).**

The present study demonstrated significant positive correlation between serum sCD40L and serum lipids in all ACS patient groups and this is in agreement with **(20)** who demonstrated that similar correlation was found between sCD40L and lipids in patients with UA and acute MI.

Conclusion:

We conclude that serum sCD40L can be used for early detection of platelet activation and release reaction in cardiac patients especially ACS to avoid risk of acute MI complications. Its determination can be used for differentiation of ACS from normal person. We recommend the use of sCD40L as a marker of platelet activation in cases of ACS. Also we recommend further studies on sCD40L in other cardiac and non cardiac diseases as early detector to decrease the risk of thrombus formation. Administration of platelet activation inhibitors is recommended in patients with UA.

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