Evaluation of Soluble Serum cd40 Ligand Level as a Significant Marker of Unstable Angina

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Abstract

The CD40L on activated T cells and platelets may be activating matrix metalloproteinases, inducing procoagulant activity, and be involved in the pathogenesis of acute coronary syndrome (ACS) by promoting plaque rupture in atheroma[1]. The aim of this work is to assess soluble serum level CD40L to clarify its role in unstable angina (UA). This work included 70 patients suffered from UA who were admitted to coronary care unit (CCU) in National Heart Institute (NHI). All subjects included in the study were evaluated by history, clinical examination, ECG, Routine biochemical investigations, CK total, CK-MB, and Quantitative ELISA for sCD40L. The sCD40L levels were significantly elevated in patients with UA when compared to controls (4.05 ± 1.61 % vs. 1.42 ± 0.63 %, P< 0.001). In multivariate logistic regression analysis, only sCD40L & FBS were efficient as predictors of UA with optimum cutoff value for diagnosis of UA was 2.15 %. Enhanced levels of sCD40L in angina patients, particularly in patients with UA, suggests that CD40L-CD40 interaction may play a role in both the long-term atherosclerotic process and in the triggering of ACS.

Keywords: sCD40L, unstable angina, acute coronary syndrome.

1. Introduction

Coronary atherosclerotic disease (CAD) is the underlying cause of UA in nearly all patients with acute myocardial ischemia. The most common cause of UA is due to coronary artery narrowing due to a thrombus that develops on a disrupted atherosclerotic plaque and is nonocclusive.[2]. CD40 was identified as a molecule expressed during all stages of B cell development and differentiation, whereas its ligand, CD40L (CD154, gp39, T-BAM, or TRAP), was mainly expressed on activated CD41 T cells. CD40-CD40L interactions play an important role in the production of several cytokines, including interleukin-12 (IL-12). Furthermore, both molecules have been described in a natural soluble form, and therefore could act at distant sites[3]. The CD40L/CD40 pathway is not only required for effective T- and B-cell immune responses but also provides a critical initial step in the development of humoral and cellular immunity [4]. Several landmark trials,
which were published in recent years, provide important insight in the therapeutic potential of anti-inflammatory strategies in atherosclerotic cardiovascular disease (CVD). Given their central role in the regulation of inflammatory processes, modulation of immune checkpoint proteins is a promising anti-inflammatory strategy for atherosclerotic CVD[5,6].

During the development of atherosclerosis, CD40 and CD40L are expressed on the majority of immune cells in the circulation and immune cells and non-immune cells within the atherosclerotic plaque. During the formation of initial plaques, CD40-CD40L interactions enhance leukocyte recruitment to the sites of vascular inflammation[7,8].

After migration into the plaque, monocytes differentiate into macrophages and secrete inflammatory effector molecules, such as cytokines and chemokines, which further propagate vascular inflammation. Genetic deficiency or antibody-mediated inhibition of CD40 (L) hampers the secretion of these inflammatory mediators, thereby limiting the differentiation of macrophages and T cells towards inflammatory M1 and Th1 subtypes. Absence of CD40 also reduces foam cell formation by limiting CD36 expression on macrophages, which may further limit lesion development. In advanced lesions, CD40-activated macrophages secrete matrix metalloproteinases (MMP), which induce plaque destabilization and rupture[9,10].

Many clinical studies have explored the potential of sCD40L as a prognostic biomarker in atherosclerotic CVD[14-11]. Although the clinical importance of circulating sCD40L in ACS is still controversial, it seems that measurement of sCD40L can be evaluated either as an inflammatory marker or as a marker of platelet activation, depending on the underlying disease state[15].

The aim of this work is to assess soluble serum level to clarify its pathogenic role in UA.

2. Patients and Methods

A written informed consent was taken from all participants after proper explanation of the study.

This is an observational case-control study that included 70 patients suffered from UA who were admitted to CCU in NHI. Thirty age and sex matched Healthy volunteers were included in the study as a control group.

All subjects included in the study were subjected to the following:
2. ECG.
3. Laboratory investigations:
   - CBC: It was done using fully automated cell counter (sysmix, Germany).
   - Routine biochemical investigations (FBS, Lipid profiles & serum creatinine) and Markers of myocardial necrosis (CK total & CK-MB): they were done using fully automated biochemical analyser (Advia 1200, Germany). The kits were provided by Siemens Advia 1200, Uk.
   - Quantitative ELISA for serum soluble CD40 Ligand: This assay employs the quantitative sandwich enzyme immunoassay technique using kits supplied by R&D Systems.

About 8 ml of venous blood was withdrawn at admission to CCU under sterile condition in a plastic syringe after discard the initial 1 ml (to avoid puncture site stimulated platelets). The blood samples were divided into four parts:
2 ml was added to EDTA at concentration of 1.2mg/ml for CBC.
3 ml was poured in serum separator tube, left to clot, and then centrifuged at high speed (1000g) for 20 minutes; serum was separated to measure routine biochemistry tests & markers of myocardial necrosis.
3 ml was poured in serum separator tube, left to clot, then centrifuged at high speed (1000g) for 20 minutes, serum was separated and stored at -20°C for quantitative ELISA for sCD40L.
2.1 Statistical analysis of the data:

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:
Independent-samples t-test of significance was used when comparing between two means. Pearson's correlation coefficient (r) test was used for correlating data. Receiver operating characteristic (ROC curve) analysis was used to find out the overall predictivity of parameter in and to find out the best cut-off value with detection of sensitivity and specificity at this cut-off value.

To identify significant independent predictors of UA, variables that were statistically significant in univariate analysis were introduced in a multivariate regression model; the overall fitness of the model was evaluated with the calculation of the coefficient R + SE. Receiver operating characteristic (ROC) curve analysis was performed to select optimal cut-off values of sCD40L.

P-value greater than 0.05 was considered as a nonsignificant result, P-value less than 0.05 was considered as a significant result, and P-value less than 0.01 was considered as a highly significant result.

3. Results

Mean age of case group was 62.81 ± 4.76 while in control group was 62.60 ± 4.97. DM was significantly higher in case group compared to control. Mean Heart rate was higher in case group compared to control. There was highly statistically significant increase in case group as regarding FBS, total cholesterol & LDL-C-c when compared to control group. There was highly statistically significant decrease in case group as regarding HDL-C-c when compared to control group.

CK total, CK-MB and sCD40L levels were significantly elevated in patients with UA when compared to controls (154.70 ± 40.62 vs. 128.20 ± 30.34, p =0.002; 20.22 ± 6.04 vs. 14.67 ± 4.45, p <0.001; 4.05 ± 1.61 vs. 1.42 ± 0.63, p <0.001 respectively) as shown in [Table 1] [Fig. 1] [Fig. 2].

In univariate logistic regression analysis, CK-MB, sCD40L, FBS, DM & HR were efficient for predicting unstable angina as shown in [Table 2]. While in multivariate logistic regression analysis, only sCD40L & FBS were efficient as predictors of UA as shown in [Table 3].

Comparison of the area under the curve for sCD40L, CK-MB and CK total reveals the fact that sCD40L may alone serve as a discriminator of patients with UA as shown in [Fig. 3].

The optimum cutoff value above which diagnosis of UA was confirmed for sCD40L was 2.15 % with sensitivity 94.29% and specificity 93.33% [Table 4].

4. Discussion

In the current study, CK total, CK-MB, and sCD40L levels were significantly elevated in patients with UA when compared to controls. This is consistent with the findings of numerous studies who found that patients with UA had significantly raised serum levels of sCD40L compared with controls [16-18]. Also, in Gururajan et al. (2009), the level of CK-MB was significantly increased in patients with ACS (62.16 ±62.78 IU/L) when compared to controls (19.47±7.31 IU/L) (P Value < 0.001) [17]. On the contrary, In Rondina et al. (2008), Median sCD40L levels were higher for patients without CAD (335 pg/ml) compared with patients with stable CAD (248 pg/ml, p = 0.01) and to patients with ACS (233 pg/ml, p <0.001). This novel interaction of sCD40L raises interesting questions for CAD pathogenesis. Although the precise mechanism is unknown, one potential theory is that sCD40L is not a marker for chronic inflammation and coronary atherosclerosis but instead is an indicator of platelet activation that is significant during
ACS but not in stable CAD[19]. In the current study, CK-MB, sCD40L, FBS, DM & HR were efficient for predicting UA. Whereas only sCD40L & FBS were efficient as independent predictors of UA. Comparison of the area under the curve for sCD40L, CK-MB and CK total reveals the fact that sCD40L may alone serve as a discriminator of patients with UA. Combined analysis of sCD40L, CK-MB and CK total may enhance prediction for UA. The optimum cutoff value above which diagnosis of UA was confirmed for sCD40L was 2.15 ng/ml. This is consistent with the findings of Gururajan et al. (2009) where the optimum cut off value from the ROC curve was 2.99 ng/ml, above which sCD40L was considered to be positive. Comparison of the area under the curve for Troponin I, CK-MB and sCD40L, reveals the fact that sCD40L may alone serve as a good discriminator of patients with ACS on comparison with gold standards. Combined analysis of sCD40L, Troponin I and CK-MB may enhance risk prediction for cardiovascular events. [17]

5. Conclusion and Recommendations:

Enhanced levels of sCD40L in angina patients, with particularly high levels in patients with UA, suggests that CD40L-CD40 interaction may play a pathogenic role in both the long-term atherosclerotic process and in the triggering and propagation of 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 CCU.

Table 1: Description and Comparison between case group and control group regarding the prognostic markers of unstable angina.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Cases group</th>
<th>Test value</th>
<th>P-value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. = 30</td>
<td>No. = 70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK total</td>
<td>Mean ± SD</td>
<td>128.2 ± 30.34</td>
<td>154.7 ± 40.62</td>
<td>-3.207*</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>80 – 180</td>
<td>80 – 220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB</td>
<td>Median (IQR)</td>
<td>15.1 (10.8 – 18)</td>
<td>20.35 (15.1 – 25.1)</td>
<td>-4.073≠</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>14.67 ± 4.45</td>
<td>20.22 ± 6.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>7.1 – 22</td>
<td>10 – 30.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD40L %</td>
<td>Median (IQR)</td>
<td>1.15 (0.98 – 1.68)</td>
<td>3.68 (2.94 – 4.99)</td>
<td>-7.402≠</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>1.42 ± 0.63</td>
<td>4.05 ± 1.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.69 – 3.16</td>
<td>1.45 – 9.63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P-value > 0.05: Nonsignificant; P-value < 0.05: Significant; P-value < 0.01: Highly significant≠: Mann-Whitney test
Table 2: Univariate logistic regression analysis for risk factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>P-value</th>
<th>Odds ratio (OR)</th>
<th>95% C.I. for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>DM</td>
<td>1.313</td>
<td>0.468</td>
<td>7.867</td>
<td>0.005</td>
<td>3.716</td>
<td>1.485</td>
</tr>
<tr>
<td>HR (beat/min) &gt;80</td>
<td>1.433</td>
<td>0.468</td>
<td>9.384</td>
<td>0.002</td>
<td>4.190</td>
<td>1.675</td>
</tr>
<tr>
<td>FBS &gt;120</td>
<td>4.584</td>
<td>1.056</td>
<td>18.835</td>
<td>&lt; 0.001</td>
<td>97.875</td>
<td>12.350</td>
</tr>
<tr>
<td>CK-MB &gt;18.1</td>
<td>1.912</td>
<td>0.519</td>
<td>13.569</td>
<td>&lt; 0.001</td>
<td>6.769</td>
<td>2.447</td>
</tr>
<tr>
<td>sCD40L % &gt;2.15</td>
<td>5.442</td>
<td>0.895</td>
<td>36.985</td>
<td>&lt; 0.001</td>
<td>231.000</td>
<td>39.982</td>
</tr>
</tbody>
</table>

B: β regression coefficient; C.I.: confidence interval; S.E.: standard error.

Table 3: Multivariate logistic regression analysis for risk factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>S.E.</th>
<th>Wald</th>
<th>P-value</th>
<th>Odds ratio (OR)</th>
<th>95% C.I. for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>FBS &gt;120</td>
<td>4.561</td>
<td>1.468</td>
<td>9.653</td>
<td>0.002</td>
<td>95.665</td>
<td>5.386</td>
</tr>
<tr>
<td>sCD40L % &gt;2.15</td>
<td>5.423</td>
<td>1.238</td>
<td>19.190</td>
<td>&lt; 0.001</td>
<td>226.608</td>
<td>20.021</td>
</tr>
</tbody>
</table>

Table 4: The cut-off value of sCD40L, CK-MB and CK total for the diagnosis of UA; ROC curve analysis.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cut off point</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+PV</th>
<th>-PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK total</td>
<td>&gt;180</td>
<td>0.690</td>
<td>32.86</td>
<td>100.00</td>
<td>100.0</td>
<td>39.0</td>
</tr>
<tr>
<td>CK-MB</td>
<td>&gt;18.1</td>
<td>0.758</td>
<td>62.86</td>
<td>80.00</td>
<td>88.0</td>
<td>48.0</td>
</tr>
<tr>
<td>sCD40L %</td>
<td>&gt;2.15</td>
<td>0.969</td>
<td>94.29</td>
<td>93.33</td>
<td>97.1</td>
<td>87.5</td>
</tr>
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</table>

Conflict of interest: The authors declare no conflict of interest.

Acknowledgement: The authors are grateful for the patients without whom this study would not have been done.

Funding sources: The authors have no funding to report

References


