Matrix metalloproteinase-9 index as a possible parameter for predicting acute coronary syndrome in diabetics

Indeks matriks metaloproteinaze-9 kao mogući parametar predviđanja akutnog koronarnog sindroma kod dijabetičara

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Abstract

Background/Aim. Matrix metalloproteinase-9 (MMP-9) index is the ratio of active MMP-9 and total MMP-9 levels. It reflects the importance of MMP-9 in acute coronary syndrome (ACS). Methods. The study included 3 groups of patients (n = 87): the group 1 – non-diabetic subjects without ACS (control); the group 2 – diabetic patients with ACS [subgroups with unstable angina pectoris (UAP), myocardial infarction (MI) or reinfarction]; and the group 3 non-diabetics patients with ACS. Total and active MMP-9 were measured and used to create MMP-9 index. Results. MMP-9 index, as a marker showed good sensitivity and specificity, of ACS in diabetics, with a cut-off value over 58.2. MMP-9 was higher in the study groups than in the control one. MMP-9 correlated with ACS occurrence and type of cardiovascular event. A statistically significant difference was found among the groups according to active MMP-9 (p < 0.001). The same was found with active MMP-9 between the control and the group with MI (p < 0.001). The control was highly statistically significantly different from the group of patients with UAP (p < 0.01). Statistically significant differences in MMP-9 index was found between the control and the diabetics with ACS (p < 0.001). Statistically significant difference of MMP-9 index was also found in the controls compared to the value in non-diabetic patients with ACS (p < 0.01). Conclusion. MMP-9 index may be a possible marker of atheromatous plaque rupture in diabetics.

Key words: diabetes mellitus; acute coronary syndrome; matrix metalloproteinase 9; prognosis.

Ključne reči: dijabetes melitus; akutni koronarni sindrom; matriks metaloproteinaza 9; prognoza.

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Introduction

Unstable atherosclerotic plaque is the pathophysiologival substrate of acute coronary syndrom (ACS)\(^1\). Enzymes matrix metalloproteinases (MMPs), especially MMP-9 secreted by inflammatory cells of atheromatous plaque (macrophages), smooth muscle cells and endothelial cells belong to a large family of zinc-binding, calcium-dependent endopeptidases that are involved in the degradation and remodeling of extracellular matrix\(^2,3\). They are a growing group of proteolytic enzymes involved in numerous processes including embryogenesis, interstitial metabolism, angiogenesis, etc.\(^4\). Matrix activity was observed in carcinogenesis, in some degenerative processes, and inflammatory conditions including atherogenesis. MMPs primarily MMP-9 has clinical significance in the serum of diabetic patients in inflammatory processes involving in plaque rupture and leading to acute coronary event with elevated levels\(^5-13\). MMP-9 is localized on the shoulders of plaque, a thin area which is suspected for rupture. The potential role of MMP-9 as a marker for risk stratification in patients with ACS was examined in a study on a larger number of patients showing that the values of MMP-9 were associated with future lethal cardiovascular events\(^6\). In patients with unstable angina pectoris (UAP) MMP-9 showed the increase of 70%, indicating active synthesis, compared with patients with stable angina. Although there is no conclusive data to show correlation of MMP with atheromatous plaques and localization of MMPs in the shoulder region of vulnerable lesions, a direct connection to the real rupture of unstable plaque has been described.

The aim of the study was to find out if the enzyme MMP-9 in the serum of diabetics and MMP-9 index could be early and safe markers of atheromatous plaque rupture and ACS.

Methods

We investigated patients admitted due to ACS to the Coronary Care Unit, Clinical Center of Serbia, Belgrade, during the period February, 2012 to February, 2013. Ethical principles were respected and all patients gave their consent to participate. A total of 87 patients of both sexes (57 male, 30 female) were examined. The patients were 40–80 years old (61.1 ± 10.3 year). The main criterion for inclusion was the presence of ACS (acute myocardial infarction or unstable angina pectoris). The two groups were formed depending on the diagnosis of diabetes mellitus (DM): diabetics with ACS (DM + ACS) and the group of non-diabetic patients with ACS. The diagnosis of ACS was based on clinical, electrocardiographic findings, biochemical analysis and diagnosis of DM based on the current American Diabetes Association criteria\(^14\). The patients with damaged hepatic function, severe anemia, neoplastic illness, infectious or autoimmune disease were excluded. DM + ACS was divided into two subgroups: UAP and the group with myocardial infarction or reinfarction (MI). The control consisted of healthy subjects (age 55.3 ± 8.9 years). MMP-9 activity was measured using detection enzymes in their pro-forms activated by binding to the active MMP-9 in the single-level enzymatic process by the method of Verheijen et al.\(^15\). The active form of MMP-9 linked to the detection enzyme was determined by binding to specific chromogenic peptide substrate complex which absorbs light of the wavelength of 405 nm. The concentration of active MMP-9 in the sample was determined by interpolation from the standard curve. Biotrak of MMP-9 activity assay system is equivalent to ELISA determination. The measuring range of the method is: the total MMP-9 1.0–32 ng/mL, and active MMP-9 0.5–16 ng/mL. MMP-9 index is defined as the relative ratio of the active and total form of MMP-9 multiplied by 100 [(MMP-9 active/MMP-9 total) × 100].

The results were reported as the mean value ± standard deviation and percentage. The differences between the groups were assessed by two-way analysis of variance (ANOVA-with Bonferroni post hoc analysis) for continuous variables and one-way non-parametric analysis of variance (Kruskal-Wallis) for category variables. Potential cutoffs for MMP-9 index were evaluated using receiver operating characteristic (ROC) curve analysis. The correlations between parameters were analyzed with Pearson's and Spearman's test. The differences were considered statistically significant at \(p < 0.05\). SPSS 12.0 software was used for statistical analysis.

Results

Table 1 shows the values of total MMP-9, active MMP-9 and MMP-9 index in the examined groups and the groups formed on coronary events.

Total MMP-9 in the examined groups

The values of total MMP-9 show a statistically significant difference among the three examined groups \((p < 0.01)\). There was a statistically significant difference of total MMP-9 between the control and DM + ACS group \((p < 0.001)\). Also, there is a statistically significant difference of total MMP-9 between the groups of diabetics and non-diabetics with ACS \((p < 0.05)\). There was no statistically significant difference of total MMP-9 between the control and the group of non-diabetics with ACS.

Active MMP-9 in the examined groups

The values of active MMP-9 showed a statistically significant difference among the three examined groups \((p < 0.001)\). The value of active MMP-9 was significantly different between the control and both groups with ACS, diabetics \((p < 0.001)\) and non-diabetics \((p < 0.001)\). The groups of diabetics and non-diabetics with ACS were statistically significantly different \((p < 0.001)\).

MMP-9 index in the examined groups

A significant difference in the values of MMP-9 index among the three examined groups was found \((p < 0.001)\), as well as between the control and DM + ACS group \((p < 0.001)\). There was a significant difference in the value of MMP-9 index in the control compared to the value in the non-diabetics \((p < 0.0)\).
Total MMP-9 in the coronary events groups

The values of total MMP-9 showed a statistically significant difference among the three groups formed on coronary events ($p < 0.05$). There was a statistically significant difference in total MMP-9 between the control group and the MI group ($p < 0.01$).

Active MMP-9 in the coronary events groups

The values of active MMP-9 show a statistically significant difference among the three groups formed on coronary events ($p < 0.001$). The value of active MMP-9 was significantly different between the control and both groups with ACS, UAP ($p < 0.001$) and MI ($p < 0.001$). The groups of UAP and MI were not significantly different in values of active MMP-9 ($p > 0.05$).

MMP-9 index in the examined groups

The values of MMP-9 index showed statistically significant difference among the three groups formed on coronary events ($p < 0.01$). The value of active MMP-9 was significantly different between the control and both coronary events groups, UAP ($p < 0.001$) and MI ($p < 0.001$). The value of MMP-9 index in the UAP group was not significantly different than the value in the MI group ($p > 0.05$).

Statistical analysis of the data presented in Figure 1 showed a significant difference in the percentage of patients with elevated total MMP-9 between the control group and the group of patients with myocardial infarction or reinfarction ($p < 0.01$).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Examined groups ($\bar{x} \pm SD$)</th>
<th>DM + ACS</th>
<th>ACS</th>
<th>Control</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MMP-9 level (ng/mL)</td>
<td>51.7 ± 13.4</td>
<td>42.2 ± 11.1</td>
<td>37.1 ± 12.4</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Active MMP-9 level (ng/mL)</td>
<td>36.8 ± 11.2</td>
<td>28.4 ± 7.3</td>
<td>16.2 ± 7.9</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>MMP-9 index</td>
<td>70.6 ± 16.8</td>
<td>68.8 ± 15.6</td>
<td>42.9 ± 10.1</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coronary events groups ($\bar{x} \pm SD$)</th>
<th>UAP</th>
<th>MI</th>
<th>Control</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MMP-9 level (ng/mL)</td>
<td>44.4 ± 15.5</td>
<td>46.7 ± 12.3</td>
<td>37.1 ± 12.4</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Active MMP-9 level (ng/mL)</td>
<td>28.9 ± 10.4</td>
<td>32.7 ± 9.9</td>
<td>16.2 ± 7.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MMP-9 index</td>
<td>66.7 ± 16.5</td>
<td>71.4 ± 16.0</td>
<td>42.9 ± 10.1</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

x – mean value; SD – standard deviation; DM – patients with diabetes mellitus; ACS – patients with acute coronary syndrome; UAP – patients with unstable angina pectoris; MI – patients with myocardial infarction or reinfarction.

Data presented in Figure 3 show a significant difference between the control group and both groups with ACS (diabetics and non-diabetics) ($p < 0.001$). The groups of diabetics and non-diabetics with ACS did not differ significantly ($p > 0.05$).
Data presented in Figure 4 show a significant difference between the control and MI group ($p < 0.001$). The control group was significantly different from the UAP group ($p < 0.01$), too.

![Figure 4](image-url)

**Fig. 4** – Distribution of elevated active matrix metalloproteinase-9 (MMP-9) levels in coronary event groups

* $p < 0.01$ compared to control; ** $p < 0.001$ compared to control; $N$ – number of patients; UAP – patients with unstable angina pectoris; MI – patients with myocardial infarction or reinfarction.

Table 2 shows the significant correlations of total MMP-9 levels and other ACS parameters. In the DM + ACS group, elevated total MMP-9 level showed the strongest correlation with the elevated Homeostatic Model Assessment of Insulin Resistance (HOMA IR) score. Elevated cholesterol levels significantly correlated with elevated total MMP-9 level in the DM + ACS group. The presence of hypertension and smoking showed a statistically significant correlation between DM + ACS with total MMP-9.

The presence of hypertension and smoking in the DM + ACS group significantly correlated with serum levels of active MMP-9.

There was a good correlation between MMP-9 index and hyperinsulinemia and pain duration in the group of diabetics with ACS.

The largest area under the ROC curve (AUC) was of active MMP-9 level, the lowest AUC was of total MMP-9 level, and AUC of MMP-9 index was between active and total MMP-9 level. The AUC of total MMP-9 level showed a significant difference ($p = 0.0041$) compared to the area of 0.5. The AUC of active MMP-9 level and MMP-9 index showed a significant difference ($p = 0.0001$) compared to the area of 0.5. AUC of total MMP-9 is significantly different from the AUC of active MMP-9 ($p < 0.05$). The AUC for MMP-9 index was not significantly different compared to the AUC of total or active MMP-9 level. The AUC of MMP-9 index had the lowest standard error (SE). The resulting cutoff value for MMP-9 index was over 58.2.

![Figure 5](image-url)

**Fig. 5** – Receiver operating characteristic (ROC) curves of total matrix metalloproteinase-9 (MMP-9) level, active MMP-9 level and MMP-9 index in diabetics with acute coronary syndrom (ACS)

Table 3 shows the values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MMP-9 level as predictor of ACS in diabetics. Total MMP-9 level had good sensitivity, but poor specificity with a high PPV and NPV. Active MMP-9 had good sensitivity and specificity, with a high PPV and NPV. MMP-9 index showed good sensitivity, excellent specificity and PPV, and acceptable NPV.

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MMP-9</th>
<th>Group</th>
<th>$r$</th>
<th>$p$</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>Total</td>
<td>All</td>
<td>0.353</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>Smoking</td>
<td>Total</td>
<td>DM + ACS</td>
<td>0.387</td>
<td>$&lt; 0.05$</td>
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<tr>
<td>Hypertension</td>
<td>Total</td>
<td>DM + ACS</td>
<td>0.469</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td>HOMAIR (high values)</td>
<td>Total</td>
<td>DM + ACS</td>
<td>0.557</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td>Cholesterol (elevated levels)</td>
<td>Total</td>
<td>DM + ACS</td>
<td>0.422</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td>Smoking</td>
<td>Active</td>
<td>DM + ACS</td>
<td>0.426</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Active</td>
<td>DM + ACS</td>
<td>0.395</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td>Insulinemia (high values)</td>
<td>Index</td>
<td>DM + ACS</td>
<td>0.542</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td>Pain duration</td>
<td>Index</td>
<td>DM + ACS</td>
<td>0.382</td>
<td>$&lt; 0.05$</td>
</tr>
</tbody>
</table>

$r$ – coefficient of correlation; DM – diabetes mellitus.

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9 total level</td>
<td>100</td>
<td>44.4</td>
<td>84.4</td>
<td>100</td>
</tr>
<tr>
<td>MMP-9 active level</td>
<td>92.6</td>
<td>88.9</td>
<td>96.2</td>
<td>80</td>
</tr>
<tr>
<td>MMP-9 index</td>
<td>77.8</td>
<td>100</td>
<td>100</td>
<td>60</td>
</tr>
</tbody>
</table>

PPV – positive predictive value of the test; NPV – negative predictive value of the test.

Discussion

The levels of MMP-9 and tissue inhibitor metalloproteinases-1 (TIMP-1) are significantly increased in coronary arteries with unstable plaques. As TIMP-1 is a potential inhibitor of MMP-9, its increase during the acute phase of MI may indicate production of MMP-9. Diabetes and/or hyperglycemia have a big influence on the structure and function of the heart and vascular tissue, inducing proinflammatory changes, including increased MMP and other factors potentially relevant to plaque rupture and thrombosis. There are studies showing no changes of MMP-9 in diabetics. Diabetes is also associated with plaque instability and carries a high risk for an acute coronary event. Sampling of MMP-9 values is related to the occurrence of acute coronary event and the diagnosis of ACS. Our results showed that the values of total MMP-9 level were higher in diabetics with ACS, than in the non-diabetics and the control. Elevated total MMP-9 level was highest in the patients with MI, then in the UAP and the control group. The mean value of total MMP-9 level in the control group was slightly above the upper limit of the reference values (37.1 ng/mL) and there was a statistically significant difference compared to the mean value obtained in the group of diabetics with ACS. The total MMP-9 level, active form, reflects the presence of ACS more precisely. The control group had statistically significantly lower values of active MMP-9 level compared to the groups of diabetics and non-diabetics with ACS. Elevated active MMP-9 level was highest in the patients with MI, then in the UAP and the control group. The number of patients with highly active MMP-9 level in the control group was highly statistically significantly different compared to the groups with MI and UAP. The mean values of active MMP-9 level between the groups of diabetics and non-diabetics with ACS were highly significantly different. In the group of diabetics with ACS, a total MMP-9 level showed the strongest correlation with the values of HOMA IR index. There was a correlation of elevated cholesterol with the elevated total MMP-9 level in diabetics with ACS. Hypertension and smoking showed a correlation with the values of total MMP-9 level in the group of diabetics. Insulin resistance is closely related to the progression of atherosclerosis and total MMP-9 level may reflect the degree of existing atherosclerosis. In the group of diabetics there was correlation between active MMP-9 level with hypertension and smoking and without HOMA IR. IR is a chronic condition, and active MMP-9 level is a parameter of acute events. A correlation was found with hypertension, also a chronic condition, but may be a sudden increase of pressure in the form of hypertensive crisis, which can trigger an acute coronary event. A correlation with smoking can be the result of the influence of carbon monoxide on the development of coronary vasoconstriction of blood vessels, which in the ground of unstable plaque and hypertension increases the turbulent flow of blood which in turn contributes to plaque rupture. This study examined the active MMP-9 level in diabetics with ACS who are particularly exposed to numerous risk factors including IR. Dysfunction in autonomic nervous system may result in pain absence during coronary event. It is necessary to predict coronary events in diabetics. The level of active MMP-9 level is associated with both plaque rupture and the massiveness of the rupture. There is no adequate information about genetic polymorphisms of MMPs. They can be partially explained by changes in the distribution of MMP-9 at the individual level. If the levels of total and active MMP-9 are directly related to the genetic polymorphism that may result in higher levels of both forms of MMP-9 without ACS. MMP-9 index reflects the impact of both forms of MMP-9. Therefore, we examined the relative ratio of active and total MMP-9, which we define as MMP-9 index. The mean value of MMP-9 index in the group of diabetics was highest, slightly lower in the non-diabetics with ACS, and lowest in the control group. MMP-9 index was very highly statistically significantly different among the groups. Our results showed that the mean MMP-9 index was slightly higher in the patients with MI than in the group with UAP but with no statistically significant differences. These results are expected because level of active MMP-9 is a marker of plaque rupture, which occurs in UAP and MI. MMP-9 index showed a statistically significant correlation with hyperinsulinemia and duration of pain in the group of diabetics with ACS. In order to prove the validity of MMP-9 index we determined sensitivity and specificity for each parameter individually (total, active MMP-9 and MMP-9 level index). Analyzing the ability of total MMP-9 level as a marker of ACS in diabetics we found an important AUC (amounted to 0.774) and the cut-off value for acute coronary events was over 27.6 ng/mL, while level of active MMP-9 in the same group the AUC was 0.936 and the cut-off value over 23.8 ng/mL. The AUC of active and total MMP-9 level differed significantly compared to the ACU of MMP-9 index. In diabetics with ACS, the AUC of MMP-9 index was 0.914 and highly significantly different compared to the AUC of 0.5. MMP-9 index cut-off value was 58.2. The AUC of MMP-9 index had the lowest values of SE.

The sensitivity of total MMP-9 level as a test for ACS in diabetics was 100%, but its specificity was low (only 44.4%). Active MMP-9 level had a good sensitivity and specificity. MMP-9 index showed a very good sensitivity and excellent specificity satisfying the requirements for screening test of ACS in diabetics. MMP-9 index is a relative number which eliminate several possible influences of the variability of active and total MMP-9 level values.

Analysing the available literature does not reveal similar results in terms of active MMP-9 level. MMP-9 index has been first postulated in this study. Total MMP-9 and active MMP-9 levels can be determined relatively quickly and easily. Their commercialization further reduces the costs and allows determination of the proposed MMP-9 index. This index could be a good marker for triage in population of diabetics and selection of candidates for elective coronary angiography. We believe that the use of this index in the secondary prevention of acute coronary events in diabetics may significantly improve cardiovascular outcomes. Without any doubt, it

is necessary to set a prospective randomized study on a larger number of patients, which may eventually correct the cut-off value of MMP-9 index determined by our study.

Conclusion

MMP-9 index could be a good marker for prediction of acute coronary syndrome in diabetic patients and for triage for elective coronary angiography.

References


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Disclosures

None of the authors have any competing interests.