Serum Levels of ICAM-1, VCAM-1 and E-selectin in early postoperative period and three months after eversion carotid endarterectomy

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ABSTRACT

Aim To determine the influence of eversion endarterectomy on circulating adhesion molecules (CAMs): E-selectin, intercellular circulating adhesion molecule-1 (ICAM-1) and vascular circulating adhesion molecule-1 (VCAM-1).

Methods Forty patients underwent carotid endarterectomy. Veno-us blood samples have been gathered before operation, one hour and six hours after the operation and three months after the procedure. Levels of CAMs have been determined by sandwich ELISA test.

Results Statistically significant decrease of the ICAM-1 levels one hour and six hours after the endarterectomy compared to levels before the operation have been found. There were no statistically significant changes in concentration of VCAM-1 and E-selectin. Three months after the operation levels of CAMs were similar to those before the operation. There was a statistically significant decrease of systolic arterial blood pressure levels within early postoperative period.

Conclusion Results suggest that decrease of ICAM-1 could be a possible marker of endothelial de-activation after plaque removal. Endarterectomy has a positive influence on systolic arterial blood pressure in early postoperative period. Further investigations are necessary to better understand and prevent the development of atherosclerotic plaque.

Key words: atherosclerosis, adhesion molecules, carotid artery stenosis

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INTRODUCTION

Geometry of carotid artery bifurcation may be one of the factors which influence hemodynamic of blood flow causing atherosclerotic plaque formation. Lateral wall of carotid artery bifurcation and lateral wall of the beginning of the internal carotid artery are predilection locations for plaque formation (1-3). Inflammation in vascular wall is an important factor of atherogenesis and early stages of atherosclerosis (4).

Cell adhesion molecules are glycoproteins situated in cytoplasm, cell membrane and there is an outer cell part. They are divided in four groups: cadherins, integrins, selectins and immunoglobulin superfamily. Cadherins connects heart cells, neural synapses and cells in myelin envelope. Integrins are involved in processes like thrombosis, morphogenesis, remodeling of tissues, leukocyte migration etc. That group includes ICAM-1 and VCAM-1. Selectins are divided to L, P and E selectin and they are very similar to each other by structure. They are named by cells where they were first discovered (L-lymphocyte, P- platelets and E- endothelial), (5). Inflammatory conditions causing increase of circulating E-selectin, ICAM-1 and VCAM-1 levels and therefore they can be used as markers of endothelial activation and deactivation (6).

Levels of soluble endothelial adhesion molecules and diabetes mellitus (DM) are positively correlated and that has been established in a number of in vitro and in vivo experiments as well as in obesity, arterial hypertension and serum lipid disorder (7-9). Haemodialysis patients have an increased level of CAMs but that is either result of inadequate clearance or enhanced synthesis and release of CAMs which has not been determined yet (10). Elevated levels of ICAM-1 were found in atherosclerotic plaques in the high-grade regions of symptomatic versus asymptomatic plaques and in high-grade versus low-grade region of symptomatic plaques (11). In a study by Hwang et al. the relationship of ICAM-1 and E-selectin with coronary heart disease (CHD) and carotid artery atherosclerosis (CAA) was independent of other known CHD risk factors suggesting that plasma levels of ICAM-1 and E-selectin may serve as molecular markers for atherosclerosis and the development of CHD (12).

Endarterectomy is a procedure where a surgeon removes atherosclerotic plaque from diseased artery. It can be done by longitudinal incision with patch plastic or dissection at the origin of internal carotid artery (ICA) and removing atherosclerotic plaque by eversion of ICA. In our research eversion endarterectomy with primary suture was an operating technique.

The aim of this paper was to determine serum levels of circulating adhesion molecules (CAMs): E-selectin, Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Adhesion Molecule-1 (VCAM-1) in patients that underwent eversion endarterectomy

PATIENTS AND METHODS

In the present study 40 patients were included and selected by Color Doppler flow imaging (CDFI) on LG Logiq E9 machine with 9 MHz probe. Stenosis was determined according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria.

Inclusion criteria were asymptomatic ≥70% stenosis of Internal Carotid Artery (ICA) CDFI determined, or symptomatic ≥50% ICA stenosis, CDFI determined, age between 51 and 81 and body mass index (BMI) less than 35 kgm−2 (Table 1).

Table 1. General descriptive parameters of patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No</th>
<th>Mean± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
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<td>169±8</td>
<td>152</td>
<td>186</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>40</td>
<td>77±10</td>
<td>58</td>
<td>99</td>
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<td>27±2</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>40</td>
<td>4.58±0.78</td>
<td>2.3</td>
<td>9.5</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>40</td>
<td>1.12±0.07</td>
<td>0.23</td>
<td>1.75</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>40</td>
<td>3.34±0.15</td>
<td>2.01</td>
<td>6.65</td>
</tr>
<tr>
<td>Tryglicerides (mmol/L)</td>
<td>40</td>
<td>2.12±0.62</td>
<td>0.41</td>
<td>7.34</td>
</tr>
<tr>
<td>Males</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Females</td>
<td>14</td>
<td>-</td>
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</tr>
</tbody>
</table>

BMI, body mass index; HDL, high density lipoproteins; LDL, low density lipoproteins;

Exclusion criteria were acute cerebral incidents within 6 weeks prior to surgery, diabetes mellitus (DM) and alcohol uptake more than 20 g per day.

All patients underwent eversion endarterectomy under local regional anesthesia with levobupivacaine hydrochloride (Chirocaine, Abbot Laboratories d.o.o., EU) 0. 25% up to 40 cc m−3 in total. The venous blood samples were taken before the procedure, one hour after procedure, six hours after the procedure and three months after surgery. All samples were then centrifuged at 2500 rpm for 10 minutes at room temperature and stored in refrigerator on -25°C. Two-step sandwich ELISA assay was used for determination of ICAM-1, VCAM-1 and E-selectin, (Human sICAM Platinum ELISA, Human sVCAM Platinum ELI-
The normality of the distribution was tested by Kolmogorov-Smirnov test and Lilliefors normality test and Skewness and Kurtosis were calculated. E-selectin variables were slightly off the normal distribution. The ANOVA for repeated measures was used as it is not very sensitive to moderate deviations from normality. Studies, using a variety of non-normal distributions, have shown that the false positive rate is not affected very much by this (Glass et al. 1972, Harwell et al. 1992, Lix et al. 1996), but inspite of that E-selectin results were confirmed with non-parametric Friedman ANOVA (13-16).

RESULTS

Levels of sICAM before the procedure were 470.54±153.84 ng/mL. One hour after the procedure concentrations dropped to 404.96±125.60 ng/mL, and six hours after to 395.39±117.87 ng/mL. Significant differences were found between the concentrations of sICAM-1 before the procedure and one hour after the procedure (p<0.001) and between the concentrations of sICAM-1 before the procedure and six hours after the procedure (p<0.001). There was no further significant decrease in period between one hour to six hours after the procedure (p=0.681). Three months after the surgery levels were similar to those before the surgery (Table 2).

| sICAM-1 before operation (ng/mL) | 470.26 | 434.35±153.84 | 263.20 | 843.48 |
| sICAM-1 1h after operation (ng/mL) | 404.96 | 396.20±125.61* | 214.66 | 771.30 |
| sICAM-1 6h after operation (ng/mL) | 395.40 | 371.53±117.86* | 235.05 | 719.51 |
| sICAM-1 3mo after operation (ng/mL) | 485.45 | 448.44±146.32* | 305.31 | 1080.5 |

*p<0.001 for values one hour and six hours after surgery

Before the procedure the Mean sVCAM-1 level was 579.54±302.63 ng/mL. One hour after the procedure the concentrations lowered to 480.98±297.26 ng/mL and then six hours after the surgery increased to 551.02±251.15 ng/mL. Three months after the procedure level of sVCAM-1 was double to those before the surgery probably due to extremely high levels measured in few patients, e.g.(Table 3), after excluding those patients from statistics, without compro-

| sVCAM-1 before operation (ng/mL) | 579.54 | 515.67±302.63 | 182.06 | 1433.30 |
| sVCAM-1 1h after operation (ng/mL) | 540.99 | 496.93±297.27 | 72.35 | 1450.00 |
| sVCAM-1 6h after operation (ng/mL) | 551.02 | 503.23±251.16 | 139.00 | 1147.80 |
| sVCAM-1 3mo after operation (ng/mL) | 1153.8 | 1000.0±629.72* | 105.77 | 2471.20 |

*p=0.001 for sVCAM levels 3 months after surgery

DISCUSSION

The aim of this paper was to determine serum levels of circulating adhesion molecules (CAMs): E-selectin, intercellular circulating adhesion molecule-1 (ICAM-1) and vascular circulating adhesion molecule-1 (VCAM-1) in patients that underwent eversion endarterectomy. For sICAM-1 the mean concentrations before the procedure dropped one hour after and six hours

Table 3. General descriptive parameters of the soluble vascular cell adhesion molecule-1 (sVCAM-1)

| sVCAM-1 before operation (ng/mL) | 579.54 | 515.67±302.63 | 182.06 | 1433.30 |
| sVCAM-1 1h after operation (ng/mL) | 540.99 | 496.93±297.27 | 72.35 | 1450.00 |
| sVCAM-1 6h after operation (ng/mL) | 551.02 | 503.23±251.16 | 139.00 | 1147.80 |
| sVCAM-1 3mo after operation (ng/mL) | 1153.8 | 1000.0±629.72* | 105.77 | 2471.20 |

*p=0.001 for sVCAM levels 3 months after surgery

Table 4. General descriptive parameters of the soluble vascular cell adhesion molecule-1 (sVCAM-1) with excluded extremely high levels in 12 patients

| sVCAM-1 before operation (ng/mL) | 579.54 | 515.67±302.63 | 182.06 | 1433.30 |
| sVCAM-1 1h after operation (ng/mL) | 540.99 | 496.93±297.27 | 72.35 | 1450.00 |
| sVCAM-1 6h after operation (ng/mL) | 551.02 | 503.23±251.16 | 139.00 | 1147.80 |
| sVCAM-1 3mo after operation (ng/mL) | 1153.8 | 1000.0±629.72* | 105.77 | 2471.20 |

*p=0.001 for values one hour and six hours after surgery

Table 5. General descriptive parameters of the soluble E-selectin (sE-selectin)

| sE-selectin before operation (ng/mL) | 49.40 | 39.76±31.11 | 13.53 | 128.51 |
| sE-selectin 1h after operation (ng/mL) | 48.25 | 37.99±26.98 | 18.59 | 113.09 |
| sE-selectin 6h after operation (ng/mL) | 47.87 | 37.34±26.99 | 18.76 | 112.66 |
| sE-selectin 3mo after operation (ng/mL) | 49.80 | 43.8±25.77 | 14.27 | 107.11 |
after the operation. Three months after the surgery levels of sICAM-1 were slightly higher than before the operation. Changes in concentrations one and six hours after the surgery were statistically significant. Changes in concentrations of sVCAM-1 in early postoperative period were not statistically significant. However, three months after the surgery average sVCAM-1 concentrations were significantly higher in comparison to the concentrations before the operation. This was a consequence of 12 extremely high values measured and after excluding those values, the change in concentration of sVCAM-1 was not statistically significant. The values of sE-selectin one hour after the procedure actually slightly decreased from those preoperatively, while after six hours and three months the mean concentration was a bit higher of those before the operation, but the change was not statistically significant.

A correlation between ischemic heart disease, peripheral arterial disease and plasma levels of CAMs has been investigated by several authors, and they all agree that there is enough evidence that inflammation plays an important role in atherogenesis but exact mechanism is not determined (17-19). Results of ICAM levels in rats after balloon injury of the carotid artery implicated that balloon injury induced or up regulated the ICAM-1 expression on vascular smooth muscle cells and on regenerating endothelial cells (20). In model on mice with apolipoprotein deficiency and deficiency of the ICAM, P and E selectin results indicated that reduction of those CAMs protects from atherosclerotic plaque forming (21). In a study on 14916 middle aged men who subsequently developed symptomatic peripheral arterial disease elevated levels of ICAM-1 have been found implicating that CAMs participated in accelerated atherosclerosis in otherwise healthy man (22). Circulating cell adhesion molecules had been shedding in circulation in acute phase response and among others that could be one of the predicting factors for cardiovascular disease (23). Researchers focused on the adhesion molecules and their part in early atherosclerosis underlines influence of the CAMs on the inflammatory cells and their presence in circulation although their origin is not fully understood. Future investigation is necessary to establish the role of the CAMs in atherosclerosis and as a possible therapeutic target because there has been a large number of polymorphism identified in the genes encoding different CAMs (24). Fassbender at al. indicates that endothelial-derived adhesion molecules levels have been elevated in patients with large brain-supplying vessels stenosis as well as in the patients with subcortical vascular encephalopathy and that they play similarly important role in cerebrovascular disease (25).

Carotid artery atherosclerotic plaque has been investigated using immunohistochemical methods. Symptomatic ones had statistically significant more ulcerations and plaque ruptures than asymptomatic patients as well as higher levels of inflammatory cells which implicated that infiltration of those cells promotes plaque rupture and subsequently embolisation and carotid artery occlusion (26). Kockx investigated specimens after carotid endarterectomy and concluded that focal intra-plaque microhemorrhages and neovascularization can promote plaque expansion and rupture after the micro vessels thrombosis (27). The research showed no evidence of association of VCAM-1 plasma levels and myocardial infarction in 474 men who suffered from MI and control group matched by age, smoking status and length of follow-up, which is in contrast with previous data about the association of ICAM-1 and myocardial infarction (28). Serum levels of ICAM-1 are reliable markers of carotid disease progression and can be used as a prognostic factor for asymptomatic patients, e.g. symptomatic patients have statistically significantly higher levels of ICAM-1 than asymptomatic ones (29).

Nuotio at al. showed no difference between plasma levels of ICAM-1 and VCAM-1 of symptomatic and asymptomatic patients in immunostaining results of fresh frozen atherosclerotic plaques specimens, which is opposite to majority of investigations (30).

Levels of ICAM-1, VCAM-1 and E-selectin have been measured in investigation of arterial lumen and neovasculature. Higher levels of CAMs on intimal neovascular than on arterial lumen endothelium have been determined and authors concluded that in pathogenesis of atherosclerosis neovascularation may play an important role (31). Measuring intima-media thickness and comparing with levels of serum CAMs authors demonstrate a positive association between those two values which further support the role of systemic inflam-
mation in the development of atherosclerotic lesion (32). Signorelli et al. tried to determine levels of plasma ICAM-1, VCAM-1 and E-selectin by comparing those levels in control group without peripheral arterial disease and peripheral arterial disease group before and after the treadmill exercise. Results confirm that CAMs increase in conditions of hemodynamic stress (33). In patients with peripheral occlusive arterial disease (PAOD) levels of VCAM-1 before the procedure and six months after percutaneous transluminal angioplasty (PTA) pointed that those patients who developed restenosis have higher levels of VCAM-1 and other markers of endothelial activation (34). The elevation of sVCAM-1 levels in 12 patients 3 months after surgery can be partly explained with probably some kind of allergic reaction on some allergens that were not present at the time of opera
tion because neither of those operated patients had CDFI determined stenosis over 30% and no local signs of inflammation, which is in accordance with a study of Chen and Khismatulin (35). It can be also explained by progression of atherosclerosis on some other place or places in circulation. Therefore, it is necessary to continue investigating this subject to be able to understand the mechanism of atherosclerotic plaque forming and then maybe in the future to be able to slow if not stop that mechanism.

The results of this study showed statistically significant decrease of ICAM-1 levels immediately and six hours after the operation. Results suggest that the decrease of ICAM-1 could be a possible marker of endothelial de-activation after plaque removal. However, lowering of the ICAMs in early postoperative period contributes with other positive
effects of endarterectomy to the benefit of the patients’ health and it remains for further research to determine if it has short or long-term influence.

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Competing interests: none to declare.

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Serumska koncentracija ICAM-1, VCAM-1 i E-selektina u ranom poslijeoperacijskom periodu i tri mjeseca nakon everzijske endarterektomije karotidnih arterija
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SAŽETAK

Cilj Utvrditi utjecaj endarterektomije na koncentraciju cirkulirajućih adhezijskih molekula, E-selektina, međustanične adhezijske molekule-1 (ICAM-1) i adhezijske molekule žilne stijenke-1 (VCAM-1).

Metode Istraživanje je provedeno na 40 bolesnika kojima je učinjena endarterektomija karotidnih arterija. Uzorci venske krvi uzimani su prije operacije, jedan sat nakon operacije, šest sati nakon operativnog zahvata, te tri mjeseca poslije endarterektomije. Koncentracija topivih adhezijskih molekula endotela određivana je sendvič ELISA testom.

Rezultati Uočeno je smanjenje vrijednosti ICAM-1 jedan sat i šest sati nakon operativnog zahvata. Promjene u vrijednostima ICAM-1 bile su statistički značajne, dok promjene koncentracije druge dvije adhezijske molekule nisu. Tri mjeseca nakon operacije vrijednosti mjerenih topivih adhezijskih molekula endotela bile su kao i prije operacije. Uočen je statistički značajan pad sistoličkog arterijskog tlaka tijekom ranog poslijeoperacijskog perioda. Nije uočena statistički značajna razlika ovisno o spolu i ITM-u.

Zaključak Pad razine ICAM-1 nakon operacije može biti pokazatelj deaktivacije endotela nakon odstranjenja aterosklerotskog plaka. Endarterektomija statistički značajno utječe na visinu sistoličkog arterijskog tlaka u ranom poslijeoperacijskom periodu. Neophodno je nastaviti ovakva istraživanja u smislu razumijevanja nastanka aterosklerotskog plaka, te mogućeg usporavanja ili zaustavljanja procesa, te prevencije cerebrovaskularnog inzulta.

Ključne riječi: aterosklerozna, adhezijske molekule, stenoza karotidne arterije