Selectins, Activated Leukocyte Cell Adhesion Molecule, and Platelet Endothelial Cell Adhesion Molecule-1 Tissue Levels in Patients With Low- and High-grade Gliomas

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Summary

Background: Adhesion molecules play an important role(s) in inflammation, tumor cell progression and invasion. Here we investigated tissue levels of selectins (E-, L-, and P-selectins), activated leukocyte cell adhesion molecules (ALCAM), and platelet endothelial cell adhesion molecules-1 (PECAM-1) in a series of patients with glial tumors and controls.

Methods: A total of 39 patients, 19 with low-grade gliomas and 20 with high-grade gliomas, and 15 deceased controls who underwent autopsy after trauma were included. Adhesion molecules were measured using enzyme-linked immunosorbent assay and the results were analyzed.

Results: Statistically significant differences were found between the patients and controls regarding every molecule (p<0.05). Furthermore, significantly higher levels were found in patients with high-grade gliomas compared with those with low-grade gliomas.

Conclusion: These results suggest that adhesion molecules may have a role in glioma formation and targeted therapies could help in the treatment of these devastating brain tumors.

Key words: Adhesion molecules, ALCAM, Brain tumors, PECAM-1, Selectin

Özet

Giriş: Adezyon molekülleri inflamasyonda, tümör hücresi progresyonunda ve invazyonunda önemli bir rol oynamaktadır. Burada, selektin (E-, L-, ve P-selektin), aktiflenmiş lökosit hücre adezyon molekülü (ALHAM) ve platelet endotel hücre adezyon molekülü-1 (PEHAM-1) doku seviyeleri glial tümörürlü hastalarda ve kontrol grubunda incelenmiştir.

Yöntem: Toplam 39 hasta (19 düşük-dereceli ve 20 yüksek-dereceli gliom) ve travma sonrası otopsi uygulanan 15 kontrol grubu çalışma dahil edilmiştir. Adezyon molekülleri ELISA yöntemi ile ölçülmüş ve sonuçlar karşılaştırılmıştır.

Bulgular: Her bir molekül düşünülüğünde kontrol ve hasta grupları arasında istatistiksel olarak anlamlı fark bulunmuştur (P < 0.05). Bunlara ek olarak, yüksek-dereceli gliomlarda düşük-dereceli gliomlara nazaran anlamlı yüksek seviyeler bulunmuştur.

Sonuç: Bu sonuçlar, adezyon moleküllerinin gliom oluşmasında rol alabileceği ve bu moleküllere yönelik ileride geliştirilebilecek hedef tedavilerin bu yönü beyin tümörlerinde yardımcı olabileceği düşünülmektedir.

Anahtar Kelimeler: Adezyon molekülleri, ALHAM, Beyin tümörleri, PEHAM-1, Selektin
INTRODUCTION

Gliomas in general are the most common brain tumors and despite modern surgery plus advanced radio and/or chemotherapy, recurrence and up-grading cannot be prevented. The majority of patients with low-grade gliomas (LGGs) and almost all patients with high-grade gliomas (HGGs), especially glioblastoma, showed up-grading within 2 to 4 years and recurrence in 12 to 15 months, respectively, even after total surgical removal. The hallmarks of a cancer are very well-known, including limitless replication, robust angiogenesis, invasion to the surrounding parenchyma, and finally metastasis (13). Recently, cancer-related inflammation was also added as a hallmark of cancer (7). Recent years showed us that adhesion molecules are involved in all of the hallmark-related cancer issues and targeting some of them provided promising results in a number of tumor types (3,32). Adhesion molecules include several members of molecules related to immunoglobulins, selectins, integrins, and mucins. Initiation of inflammation, cell-cell adhesion, interaction with the extracellular matrix, invasion of the surrounding tissues, and distant metastasis by blood or the lymphatic system are all processes in which adhesion molecules are involved.

In this prospective study, we wanted to present our results related to tissue levels of selectins (E-, L-, and P-selectins), activated leukocyte cell adhesion molecule (ALCAM) and platelet endothelial cell adhesion molecule-1 (PECAM-1) in patients with low- and high-grade gliomas and to show whether there was a difference between these two tumor types and controls.

MATERIAL AND METHODS

This study was performed with the collaboration of the Departments of Neurosurgery and Biochemistry. Patients who had histopathologically proven LGG (mainly grade-II) and HGG (grade-III and IV) and who signed the informed consent were included. All patients or next of kin were fully informed and ethical approval for this study was obtained from the local ethics committee.

Patients

A total of 39 patients served as subjects in this study. The LGG group included 19 adult patients (9 women and 10 men) with a mean age of 34.3 years. The majority of the patients in this group was admitted to us mainly due to headache (12 patients), followed by seizure (7 patients), and magnetic resonance imaging (MRI) of the head showed a space-occupying lesion on the right in 9 and on the left in 10 patients. The frontal lobe was the most commonly involved lobe (12 patients), followed by temporal (4 patients), insula (2 patients), and parietal lobes (1 patient). Surgical removal was performed in all and histopathologic diagnosis was grade-II (18 patients) and grade-I (1 patient) glioma.

Twenty patients (9 women and 11 men) were included in the HGG group and the mean age was found as 48.6 years. The main symptom was seizure (7 patients), followed by headache (6 patients), feeling of hypoesthesia on one side (3 patients), nausea/vomiting (1 patient), and three patients were admitted because of recurrence. Head MRI showed high-grade lesions in 12 patients on the right side, and 8 on the left. As in the LGG group, the frontal lobe was the most frequently involved lobe (12 patients), followed by parietal (4 patients), occipital (2 patients), insula (1 patient), and temporal lobes (1 patient). Following surgery, histopathologic diagnosis was grade-III glioma in 7 patients and grade-IV in 13.

Controls

The control group comprised 15 people who died in traffic accidents or by falling from height; all underwent autopsy procedures in the Department of Forensic Medicine. No subject showed gross
pathology in the brain during the autopsy procedures.

**Specimen handling**

An adequately-sized tumor sample was removed from each patient before thermal coagulation in the operating room and taking particular care to remove specimens from the core of the tumors. Cerebral tissues were removed during the autopsy procedures, which were performed within four hours of the insult. Each sample was stored at -80°C as quickly as possible until required for assay.

**Biochemical Assays**

**Preparation of tissue samples**

Tumors and control cerebral tissues were washed in cooled 0.9% NaCl and placed on an ice-cold plate, incised, and weighed. The samples were then immediately frozen in liquid nitrogen until they were homogenized. Tissue samples were homogenized manually in homogenizing buffer (100 mM KH2PO4K2HPO4) to obtain 20% homogenates using a tissue grinder fitted with a Teflon pestle for the measurement of adhesion molecules' levels, and for total protein determination. The homogenates were sonicated using an MSE sonicator two times at 30-second intervals on ice, with a power output of 38 watts. The supernatant fractions of tissue homogenates were divided into aliquots (one for each assay) and immediately stored at -80°C for analysis. The sonicated homogenates were centrifuged at 15,000 g for 15 minutes for the measurement of E-, L-, and P-selectins (CD62P), ALCAM, sPECAM-1, and protein content. The biochemical assays were performed in the supernatants.

**Assay of adhesion molecule levels**

Human endothelium selectin (E-selectin), human leukocyte selectin (L-selectin), human platelet selectin (P-Selectin, CD62P), human activated leukocyte cell adhesion molecule (ALCAM), and human soluble platelet endothelial cell adhesion molecule-1 (sPECAM-1) levels were measured using commercially available enzyme linked immunosorbent assay (ELISA) kits (YH Biosearch Laboratory, Shanghai, China) based on biotin double antibody sandwich technology. The actual levels of adhesion molecules in the samples were determined using standard curves. All molecules studies here are provided as ng/mg protein.

**Measurement of serum total protein content**

Total protein content of the serum samples was measured using the modified method of Biuret with some volumetric modifications, as proposed by Ithazaki and Gill(15). The principle of the method is based on the formation of a Cu2+-protein complex production, a violet-colored chelate product, which can be measured using absorption spectroscopy at 540 nm. Biuret reagent was prepared by adding 3 g of CuSO4.5H2O and 9 g of sodium potassium citrate to 500 mL of 0.2 N NaOH, followed by the addition of 5 g of KI. Biuret reagent was added to all samples and standards at a volume of 1:20. After a 20-minute incubation period, colorimetric reading was performed for all specimens. Recorded absorptions of the samples at 540 nm were compared with the protein standards.

**Statistical Analysis**

We used a commercially available statistical software package (SPSS version 14.0 Inc., Chicago, IL, USA) for all statistical analyses. The mean ± SD was calculated for each parameter. For all comparisons with respect to measurements of the molecules, the non-parametric Mann-Whitney U test was used as a statistical method. Differences were considered statistically significant if p<0.05.

**RESULTS**

A summary of the statistical comparisons for each group is provided in Table 1 and Table 2 and figures (Figure 1 through
Figure 3) show graphical representations of the mean levels with respect to each molecule in each group. The mean levels of the molecules, with the exception of ALCAM in the LGG group, were higher than those of the controls and the differences were significant (p< 0.001). The mean ALCAM tissue level in the controls was higher than in the LGG group (0.26 ± 0.3 versus 0.21 ± 0.1) and the difference was found significant (p=0.003). In addition, the mean level for each molecule in the LGG group was found lower than that of the HGG group and the differences were significant (p< 0.05). Considering the mean levels of the molecules between the controls and HGG group, very high levels were found in HGG and the differences were statistically significant (p< 0.001). Different from the LGG group, the mean tissue ALCAM level showed a significantly higher level in the HGG group compared with the controls (P = 0.003). We also wondered whether the mean levels of each molecule showed differences according to the grade of glioma (Table-2). In the LGG group, 18 of the 19 patients were grade-II, and 7 in the HGG group had grade-III and 13 had grade-IV (GBM). Both grade-III and -IV showed higher levels of all adhesion molecules compared with grade-II, and the differences were statistically significant (p < 0.05). However, grade-III and -IV showed levels very close to each other and comparisons between the two groups within the HGG group showed no statistically significant differences (p>0.05).

Figure 1: Graphical representations of selectin family, namely E- (a), L- (b), and P-selectin (c) in the controls and patients. The circle in the error bars represents the mean levels of each molecule. The difference between the controls and each patient group showed a statistically significant difference (p<0.05). As the grade increased, the difference also reached significant level.
Figure 2: Activated leucocyte cell adhesion molecule (ALCAM) showed significantly higher mean levels in high-grade gliomas compared with low-grade gliomas (p<0.05) but the controls had significantly higher mean levels compared with low-grade gliomas (p<0.05).

Figure 3: In this graphical representation, platelet cell adhesion molecule-1 (PECAM-1) clearly showed significant higher mean levels in both low- and high-grade gliomas compared with the controls (p<0.05). Furthermore, high-grade gliomas showed significantly higher mean levels compared with low-grade gliomas (p<0.05).
Table-1. *Summary of statistical comparisons (mean ± SD) of the studied adhesion molecules (ng/mg protein).

<table>
<thead>
<tr>
<th>Groups</th>
<th>E-selectin</th>
<th>L-selectin</th>
<th>P-selectin</th>
<th>ALCAM</th>
<th>PECAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGG</td>
<td>1.63 ± 0.1</td>
<td>42.68 ± 0.9</td>
<td>123.8 ± 11.8</td>
<td>0.21 ± 0.1</td>
<td>24.38 ± 2.7</td>
</tr>
<tr>
<td>HGG</td>
<td>1.92 ± 0.3</td>
<td>49.70 ± 5.5</td>
<td>157.7 ± 13.1</td>
<td>0.29 ± 0.1</td>
<td>27.76 ± 3.0</td>
</tr>
<tr>
<td>Controls</td>
<td>0.68 ± 0.1</td>
<td>19.87 ± 2.9</td>
<td>96.07 ± 13.9</td>
<td>0.26 ± 0.3</td>
<td>18.90 ± 2.3</td>
</tr>
<tr>
<td>LGG vs. HGG</td>
<td>&lt; .001</td>
<td>.001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>LGG vs. C</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>.003</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>HGG vs. C</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>.003</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

ALCAM: Activated leukocyte cell adhesion molecule; C. Controls; PECAM-1: Platelet endothelial cell adhesion molecule-1; HGG: High-grade glioma; LGG: Low-grade glioma; SD: Standard deviation. *P was accepted as statistically significant if less than 0.05 and the Mann-Whitney U test was used.

Table-2. *Summary of statistical comparisons (mean ± SD) of the adhesion molecules (ng/mg protein) according to grade.

<table>
<thead>
<tr>
<th>Grade/No</th>
<th>E-selectin</th>
<th>L-selectin</th>
<th>P-selectin</th>
<th>ALCAM</th>
<th>PECAM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>II/18</td>
<td>1.63 ± 0.1</td>
<td>42.9 ± 4.9</td>
<td>123.4 ± 12.0</td>
<td>0.22 ± 0.04</td>
<td>24.4 ± 2.78</td>
</tr>
<tr>
<td>III/7</td>
<td>1.92 ± 0.2</td>
<td>49.4 ± 5.8</td>
<td>159.5 ± 16.6</td>
<td>0.29 ± 0.05</td>
<td>29.1 ± 1.28</td>
</tr>
<tr>
<td>IV/13</td>
<td>1.92 ± 0.4</td>
<td>49.8 ± 5.5</td>
<td>156.7 ± 11.7</td>
<td>0.28 ± 0.03</td>
<td>27.0 ± 3.4</td>
</tr>
<tr>
<td>II vs. III</td>
<td>.001</td>
<td>.018</td>
<td>&lt; .001</td>
<td>.004</td>
<td>.001</td>
</tr>
<tr>
<td>II vs. IV</td>
<td>.003</td>
<td>.004</td>
<td>&lt; .001</td>
<td>.001</td>
<td>.022</td>
</tr>
<tr>
<td>III vs. IV</td>
<td>.93</td>
<td>.87</td>
<td>.50</td>
<td>.40</td>
<td>.12</td>
</tr>
</tbody>
</table>

ALCAM: Activated leukocyte cell adhesion molecule; C. Controls; PECAM-1: Platelet endothelial cell adhesion molecule-1; SD: Standard deviation. *P was accepted as statistically significant if less than 0.05 and the Mann-Whitney U test was used.

DISCUSSION

There have been several reports regarding adhesion molecules and their roles in pathologic processes. They are involved in the process of inflammation, which is the basis of diseases including cancer in any organ (8,25). Here, we reported tissue levels of some key adhesion molecules including the selectin family, and ALCAM and PECAM-1, in glial tumors, which have been reported to be involved in several central nervous system diseases, in addition to the role(s) they play in brain metastasis (5). However, there are very limited reports about the levels of these molecules in glial tumors.

The selectin family includes three members of adhesion molecules, namely E-, L-, and P-selectins, and they are carbohydrate-binding type-I transmembrane glycoproteins (17). E-selectin or endothelial selectin (CD62E) is constitutively expressed by the skin and bone marrow endothelium after local activation (22). Inflammatory stimuli induce E-selectin expression within 2-6 hours after stimulation and maximal expression can be seen within 6-12 hours in the liver, lung, and brain (35). L-selectin, also known as leukocyte selectin (CD62L), is also constitutively expressed by leukocytes and plays a role in the tethering and rolling of leukocytes on the endothelium as a response to inflammation (16). P-selectin or platelet selectin (CD62P) is stored in the alpha granules of platelets and Weibel-Palade bodies of endothelium and upon activation, and is released through exocytosis (22). It should
be noted that there is a delay before P-selectins appear on the cerebral endothelium, Weibel-Palade bodies of which do not contain P-selectin whose mRNA needs to be translated upon activation by inflammation (2). Selectin-mediated adhesion of inflammatory cells, mainly leukocytes, needs interaction with their ligands. The most important selectin ligand is P-selectin glycoprotein ligand-1 (PSGL-1), which is also found on the microvilli of leukocytes (17). All three members of the selectin family bind to this ligand, further assisting tethering, rolling, and extravasations of leukocytes to the diseased area.

Selectins in general have not been studied in brain tumors and the majority of studies focused on the role of selectins in metastasis. E-selectin has been shown as a supporter of metastasis in vivo (8,22). Expression of E-selectin on cancer cells enhances adhesion to activated endothelium (14). Furthermore, binding of E-selectin on cancer cells may lead to changes in the gene expression profile of a cancer, which increases the survival of cancer cells (31). It has been demonstrated that leukocytes promote tumor growth and metastasis (8,22). L-selectin mediated leukocyte recruitment plays a role in this respect because L-selectin expression is restricted to leukocytes (27). Experimental studies showed that absence of L-selectin attenuated metastasis (23). Substantial evidence indicates that platelet P-selectin contributes to the formation of thrombi and may help local colonization of cancer cells (18). Inhibition of P-selectin-mediated interactions of platelets with cancer cells diminishes metastasis in mice (4).

Activated leukocyte cell adhesion molecule (ALCAM), also known as CD166, is a member of the immunoglobulin superfamily and expressed on activated leukocytes, fibroblasts, neurons, and endothelial cells (36). It may be involved in angiogenesis, neurogenesis, and leukocyte trafficking, and hematopoietic stem cell maintenance in bone marrow (6). Recent reports showed that ALCAM was expressed at higher levels in some types of cancers and was a marker for prostate and colon cancers (9,33). The function of ALCAM is not well known, but it functions as a cell surface sensor for cell density in metastatic melanoma and helps tumor cell proliferation and invasion (11). It is believed that ALCAM triggers the activation of the metalloproteinase cascade for cell-to-matrix contacts (24). Although studies including glial tumors regarding ALCAM are very rare in the current literature, a recent study demonstrated that ALCAM was expressed on glioblastoma progenitor cells, and was involved in the regulation of glioblastoma invasion and progression (20). Expressions of ALCAM (membranous or cytoplasmic) have been shown in different types of cancers other than brain tumors, and different results were obtained. In some tumor types, higher expressions were correlated with poor prognosis, but in others, loss or lower levels were correlated with more aggressive phenotype or poor prognosis (21,29,34,37). This was explained by fact that membranous versus cytoplasmic expressions of ALCAM may show different effects on tumor cells (38). Another important function of ALCAM is that it is localized at the cell-cell junctions in endothelial cells; therefore, it may have a role as a potential anti-angiogenesis target (38). ALCAM antibodies are able to block transendothelial migration of activated monocytes, which indicates that ALCAM may control diapedesis (28,38).

Platelet endothelial cell adhesion molecule-1 (PECAM-1) or CD31, like ALCAM, is an adhesion molecule that belongs to the immunoglobulin superfamily. It is a transmembrane molecule and expressed on platelets, monocytes, and neutrophils (30). PECAM-1 is also highly expressed on endothelial cells where it plays a role in vascular membrane integrity (30). During
inflammation, PECAM-1 also leads to extravasation of leukocytes it functions with the collaboration of ALCAM in this process. The most important proven function of PECAM-1 so far is its role in angiogenesis, which is one of the hallmarks of cancer (12). Blockade of PECAM-1 showed inhibition of angiogenesis in animal models (10). A limited number of clinical studies including low- and high-grade gliomas showed that PECAM-1 expression levels were correlated with malignancy. The highest results were found in glioblastoma followed by anaplastic astrocytoma (1,19,26).

Overall, the aforementioned data indicate that adhesion molecules may play an important role in inflammation, cancer cell metastasis, and angiogenesis, which is sine qua non for cancer progression and invasion. Thus, understanding the pathophysiologic mechanism(s) leading to the development of cancer is of utmost importance, and inhibition of the functions of adhesion molecules could help to decrease the progression of these devastating brain tumors. Our study is the first to show expression(s) of selectins, ALCAM, and PECAM-1 together on both low- and high-grade gliomas. Our findings are in line with the limited literature in which HGGs showed higher expressions of these molecules compared with both controls and LGGs. We expected to have the highest level of molecules in HGGs, especially in glioblastoma, which is the most aggressive brain tumor. Thrombosed vessels in high-grade gliomas were the main surgical finding, which may be due to the higher expressions of P-selectin. Severe edema is another finding observed on MRI that threatens patients' lives in case of midline shift. This finding is related to the induced angiogenesis by new vessels sprouting from the preexisting host vessels. Newly formed vessels are weak and fenestrated which, leads to leakage. In this respect, higher levels of ALCAM and especially PECAM-1 in the present study can explain edema seen every patient with HGG compared with LGG. A statistically significant difference between the tumors and controls was expected in light of the current literature, and with the exception of ALCAM, our findings between the LGGs and controls support the current data. The higher mean level of ALCAM in the controls compared with the LGGs was not expected. One reason for this unexpected finding may be that inflammation after acute trauma is more severe than in inflammation caused by an LGG, which is a chronic tumoral lesion. Comparisons according to the grade of the tumors in the present study showed that grade-III and -IV had higher levels of adhesion molecules compared with grade-II. This indicates that the higher the grade, the higher the levels of adhesion molecules, which may explain the aggressiveness of HGGs. Interestingly, we found no statistically significant difference between grade-III and -IV in which the levels were close to each other for every molecule studied. This may be because grade-III glial tumors may be as aggressive as grade-IV glial tumors or even more so, which is something neurosurgeons encounter in the clinical setting both clinically and radiologically. This means that we have to be alert and steadfast in the treatment of grade-III tumors as is the case with patients who have grade-IV glial tumors (glioblastoma multiforme).

In conclusion, selectins, ALCAM, and PECAM-1 are highly expressed in glial tumors and the levels tend to increase as the tumor grade increases. Targeted therapies in the future may help physicians treat these devastating lesions.

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Tumor samples were re-used here for the extension of our previous work.
The authors declare no conflict of interest.
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