Paraoxonase and Prolidase Activity in Patients With Malignant Gliomas

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Summary

Aim: We aimed to determine the relationship both paraoxonase and prolidase activities in the patients with malignant glioma.

Methods: In this study, serum paraoxonase and prolidase activities were measured on 25 healthy human and 25 patients with malignant glioma. Paraoxonase activity was measured using diethyl-p-nitrophenylphosphate as substrate, as previously described. The prolidase activity was used for measurement of proline by the method proposed by Myara et al., which is a modification of Chinard's method (Myara et al.,1982).

Results: The paraoxonase and prolidase activities were significantly lower in serum of patients with malignant glioma as a whole compared to controls.

Conclusions: In the current study, the activity paraoxonase and prolidase decreased in the patients with malignant glioma. As a result the paraoxonase and prolidase decreased seems to play a major role in the pathophysiology of malignant glioma. This is the first report on serum paraoxonase and prolidase decreased activities in patients with malignant gliomas.

Key words: Malignant glioma , paraoxonase activity , prolidase activity

Özet

Amaç: Biz malign gliomlu hastalarda hem paraoxonaz (PON), hemde prolidaz (PR) aktivitesi arasında ki ilişkiyi belirlemeyi amaçladık.

Metodlar: Bu çalışmada 25 malign gliomlu hasta ile 25 sağlıklı kontrol grubunda serum PON ve PR düzeyleri ölçüldü. PON aktivitesi daha önceki çalışmalarında olduğu gibi, substrat olarak dietil-p-nitrofenil fosfat kullanılarak ölçüldü. PR aktivitesi Chinard's metodunun (Myara et al.,1982) bir modifikasyonu olan Myara ve ark.ın önerdiği prolinin ölçülmesiyle ortaya konuldu.

Bulgular: Kontrol grubuyla karşılaştırıldığında malign gliomlu hastalarda serum PON ve PR aktivitesi önemli derecede düşük bulundu.


Anahtar Kelimeler: Malign gliom,paraoxonaz aktivitesi, prolidaz aktivitesi
INTRODUCTION
Glioma is a broad category of brain tumors that result from glial cells. Gliomas are the most common intra-axial tumors arising from the central nervous system. They are graded from low to high grade tumors by their histologic appearance and grade 3 and 4 are considered malignant glioma\(^{(2,6,14)}\). The malignant gliomas tend to be faster growing, more aggressive and more invasive into the surrounding brain tissue. Anaplastic astrocytoma and glioblastoma are the most malignant and aggressive form of gliomas which have very poor prognosis\(^{(2)}\).

Prolidase (PR) \([\text{EC.3.4.13.9}]\) is a cytosolic imidodipeptidase, which specifically splits imidodipeptides with C-terminal proline or hydroxyproline\(^{(11)}\). PR is widely distributed in man and animals\(^{(13)}\). This activity is relatively high in kidney, intestinal mucosa, and erythrocytes, and low in liver and plasma, prolidase activity in plasma being as great as about \(\%\) 6 in erythrocytes\(^{(17)}\). Clinically, deficiency is characterized by dermatological symptoms, especially severe leg ulcers, and different degrees of mental retardation\(^{(19)}\).

Paraoxonase (PON) is a calcium dependent glycoprotein that is associated with high density lipoprotein (HDL)\(^{(10)}\). Isoforms of PON are widely available in many tissues of animals such as kidney, liver, small intestine and also serum\(^{(4,16)}\). PON activity is significantly decreased in lung cancer patients compared to healthy controls\(^{(9)}\).

The purpose of this study is to investigate the PR and PON activity in malignant glioma due to might help to elucidate the pathophysiology.

MATERIAL AND METHODS
Biochemical Analysis
25 patients with malignant glial tumors were operated in Neurosurgery Clinic of Yuzuncu Yil University between January 2007 to July 2010. The age of patients ranged between 45-75 (mean 60). 16 (64 \%) of these patients were diagnosed as glioblastome multiforme and 9 (36 \%) were diagnosed as anaplastic astrocytoma in histopathologic examination. As control group were included 25 healthy human. Venous blood samples of the patients with malignant glioma radiologically diagnosed with magnetic resonance imaging (MRI) were obtained before operation and diagnosis was confirmed histopathologically after surgery. Venous blood samples of malignant glioma were obtained from the antecubital fossa vein in patient with malignant glioma. Informed consent was obtained from all participating subjects before the initiation of the study which was carried out according to the rules of the Declaration of Helsinki. Serum was separated by centrifugation and the samples were processed immediately. The serum samples were placed in deionised polyethylene tubes and kept at -80oC in a deep-freeze (without thawing) until the day of study.

Determination of PON and PR activities
PON activity was measured using diethyl-p-nitrophenylphosphate as substrate, as previously described\(^{4,8,11}\). Assays were made either without additional NaCl (baseline activity) or with 1 M NaCl included in the assay buffer (salt-stimulated activity), following the formation of p-nitrophenol by its absorbance at 405 nm for 3 min. Enzymatic activity was calculated using the molar extinction coefficient 18 000 M\(^{-1}\) cm\(^{-1}\). One unit of paraoxonase activity was defined as the enzyme quantity that disintegrates 1 \(\mu\)mol paraoxon substrate in one minute. The PR activity was expressed by the measurement of proline which was proposed by Myara et al., a modification of Chinard's method\(^{(5,17,18)}\).

RESULTS
The SPSS (ver. 13) statistical program was used for all statistical computations.
Statistical Analysis Descriptive statistics for studied variables (characteristics) were presented as mean, standard deviation, minimum and maximum values. Student-t test was used to compare control and patient group means for the studied variables. Cut off value of PON and PR were determined by ROC analysis. Statistical significance levels were considered as p<0.05.

The PON activity was significantly lower in level of serum of patients with malignant glioma as a whole compared to controls (P<0.05) (Table 1). The serum PR activity was found to be decrease significantly in patients group compared to control group (P<0.05). The cut off value for PON and PR was 104.69 and 0.99, respectively. Their specificity and sensitivity was % 100.

Table 1. Descriptive statistics and comparison results for groups.

<table>
<thead>
<tr>
<th></th>
<th>Patients with gliomas (n=25)</th>
<th>Control group (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>54.0 ± 4.3</td>
<td>52.7 ± 6.1</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Paraoxonase (U/ml)</td>
<td>70.68±17.03</td>
<td>131.13±14.07</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Prolidase(U/ml)</td>
<td>0.74±0.12</td>
<td>1.37±0.29</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>111.3 ± 29.8</td>
<td>123.3 ± 40.2</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>36.9 ± 11.4</td>
<td>32.5 ± 7.7</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.84 ± 0.14</td>
<td>0.82 ± 0.19</td>
<td>P&gt;0.05</td>
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DISCUSSION

Gliomas arise from the supportive tissue of the brain. There are several different types of gliomas. The type of glioma is determined by the cells that give rise to the tumor. Anaplastic astrocytoma, and glioblastoma are two examples of gliomas. These consist of at least 35% of primary brain tumors and their incidence are 3/100,000(6,14).

PR is a cytosolic enzyme, necessary for specific splitting of imidodipeptides with proline or hydroxyproline at their C-terminals. PR is a member of the matrix metalloproteinase family. It plays a major role in collagen turnover, matrix remodeling and cell growth(12). The enzyme plays an important role in the recycling of proline from imidodipeptides (mostly derived from degradation products of collagen) for resynthesis of collagen and other proline-containing proteins. The enzyme activity is up-regulated by β1-integrin receptor stimulation. The increase in the enzyme activity is due to its
phosphorylation on serine/threonine residues\(^{21}\). PR has a major role in collagen turnover and cell growth\(^7,20\). Its activity has been documented in plasma, erythrocytes, leukocytes, dermal fibroblasts, and in various organs, such as kidney, brain, heart, thymus, uterus, liver, small intestine, stomach, spleen, lung, and pancreas\(^{15,23}\). The investigations on serum PR activity showed that it changes in various diseases such as liver cirrhosis and breast cancer\(^{22}\). Serum PR activity was reported to be increased in patients with hypertension\(^{7}\). We performed serum PR activity, with colorimetry of proline by Chinard's method\(^5\). Serum PR activity was lower in malignant glioma than in control group, and the differences there are reach a statistical significance. Also, in our study, the cut off value for PR was 0.99 and its specificity and sensitivity was found to be 100%. We assume that if value of PR is beneath 0.99, it may be possible that person is at risk for malignant glioma.

PON is a glycoprotein enzyme with 354 amino acids. The results of recent studies investigating the association between serum PON activity and various forms of cancer are highly variable. Serum levels of PON were reported to be lower in patients with pancreatic and gastric cancer than healthy controls in two case control studies\(^1\). Oxidative stress and inflammation are believed to be important in carcinogenesis but there were only a few studies on changes in PON activity in cancer patients\(^8\). A previous study reported an increase in oxidative stress and a decrease in paraoxonase activity and also down regulation of PON expression\(^3\). In addition, PON activity has been reported higher in esophageal cancers\(^4\). The relevance of the relationship between PON and brain tumors is not shown, because despite these studies, there are few data on the activity of PON in brain tumors. Therefore, determination of PON enzyme activity is important in brain tumors. Therefore, we decided to measure the PON activity. Data of our study showed that PON activity was lower in patient group than in control group. In the current study, the cut off value for PON was 104.69 and its specificity and sensitivity was % 100 for malignant glioma. If value of PON is beneath 104.69, it may be possible that person is at risk for malignant glioma. We found that serum level of PR and PON have an important role in forming of malignant glioma.

As other cancer types, some prognostic factors can be used in malignant gliomas. These factors can be used, especially after surgery, treatment and follow-up. Our results suggest that serum PON and PR as a part of the enzyme activity systems might be involved in the tumourigenesis of brain tumours. PON and PR may be a marker of malignant glioma. On the other hand, reviewing the literature this study is the first report on serum PON and PR activities in patients with malignant gliomas.

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**Received by:** 01 October 2012
**Revised by:** 08 November 2012
**Accepted:** 09 November 2012

**The Online Journal of Neurological Sciences (Turkish) 1984-2012**
This e-journal is run by Ege University Faculty of Medicine, Dept. of Neurological Surgery, Bornova, Izmir-35100TR as part of the Ege Neurological Surgery World Wide Web service.

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Journal of Neurological Sciences (Turkish)
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