**BIOCHEMICAL STUDY ON SUPEROXIDE DISMUTASE ENZYME IN PATIENTS WITH DIFFERENT BRAIN TUMORS**

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**ABSTRACT**

The aim of the current study is to evaluate the role of SOD activity in the previously reported oxidative stress in our laboratory (1), in the patients with different brain tumors. SOD activity was assayed according to riboflavin/NBT method and its specific activity was calculated in patients with benign and malignant brain tumors and control. Moreover the specific activity was compared in these samples according to gender and the occurrence of disease. Non-significant elevation (P > 0.05) in SOD specific activity was observed in tissue of malignant tumors in comparison to that of in benign brain tumors. While a highly significant decrease (P < 0.001) of the specific activity was found in sera of malignant patients group in comparison to that of the control group, and it was found lower in female than male in control and malignant groups. An elevation in this specific activity was noticed in patients with secondary brain tumors in comparison to that of primary brain tumors (P<0.05). From the results of the present study we conclude that the observed decrease in SOD activity in sera of patients with different type of brain tumors contribute to the oxidative stress that previously reported in our laboratory to be present in such patients.

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Key words: Brain tumor, Superoxide dismutase, Reactive oxygen species.
دراسة كيموحيوية لانزيم superoxide dismutase

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الخلاصة

الهدف من هذه الدراسة هو تحديد دور فعالية انزيم SOD في الجهد التأكسدي والذي سبق وان سجل وجوده في أنسجة ومصل الدم من قبل الباحثين في مختبرنا في المرضى المصابين بأورام الدماغ المختلفة. لقد تم تقييم الفعالية النوعية لهذا الإنزيم في المرضى المصابين بالأورام الدماغ الخبيثة والحميدة والأصحاء، فضلا عن ذلك فقد تم مقارنة الفعالية النوعية في المجموعات المدروسة باعتماد طريقة riboflavin/NBT لتقدير فعالية إنزيم SOD. أظهرت النتائج ارتفاع غير ممروس في الفعالية النوعية لإنزيم SOD في أنسجة المصابين بأورام الدماغ الخبيثة الحميدة، وفي حين لوحظ انخفاض ممروس جدا في هذه الفعالية النوعية في مصل المصابين بالأورام الدماغ الخبيثة الحميدة والأصحاء، وعند مقارنة الفعالية النوعية لهذا الإنزيم في مصل المصابين بالأورام الدماغ الخبيثة الحميدة بالأصحاء، وを利用して النتائج ارتفاع غير ممروس في فعالية الإنزيم SOD في المصابين بالأورام الدماغ الخبيثة الثانوية وعند مقارنة الفعالية النوعية لهذا الإنزيم بين الأصحاء والمصابين بالأورام الدماغ الخبيثة الثانوية. تظهر النتائج وجود زيادة فيها في مصل أولئك المصابين بالأورام الدماغ الخبيثة الثانوية، وعند مقارنة الفعالية النوعية لهذا الإنزيم بين المصابين بالأورام الدماغ الخبيثة الخبيثة الثانوية وعند مقارنة الفعالية النوعية بين المصابين بالأورام الدماغ الخبيثة الخبيثة الثانوية والأصحاء، وعند مقارنة الفعالية النوعية بين المصابين بالأورام الدماغ الخبيثة الخبيثة الثانوية والصحة.

كما وجدت أن الفعالية النوعية لهذا الإنزيم منخفضة في النساء مقارنة بالرجال، ومن المصابين بالأورام الدماغ الخبيثة الثانوية، وعند مقارنة الفعالية النوعية لهذا الإنزيم بين المصابين بالأورام الدماغ الخبيثة المبكرة والأورام الدماغ الخبيثة الثانوية.

الخلاصة من نتائج الدراسة الحالية إن الانخفاض المجهوظ في فعالية الإنزيم SOD في مصل المرضى المصابين بأورام الدماغ يساهم في الجهاد التأكسدي الذي سبق وان سجل عند هؤلاء المرضى في مختبرنا.
INTRODUCTION

The brain is exposed throughout life to oxidative stress (OS), neurons are highly sensitive to OS(1), and certain disease of the brain and nervous system are thought to involve free radical process and oxidative damage, either as a primary cause or as a consequence of disease progression(2). Cells differ profoundly in their resistance to such stress.Superoxide dismutase (SOD), which is present in different isoenzyme, is the major antioxidant enzyme(3), which scavening superoxide ion by speeding up its dismutation(4).

A free radical is defined as any molecule or atom, which contains one or more unpaired electrons in its outer orbit (5). The most important radicals in biological systems are the superoxide anion (H2O2), hydroxyl radical (OH˙), nitric oxide (NO˙), the lipid-derived peroxyl radical (ROO˙), and alkoxyl radical (RO˙). These radicals were called "Reactive Oxygen Species" (ROS). Reactive oxygen species are potentially harmful, because they interact with each other and modify cellular biomolecules(6) as shown in Figure (1):

ROS can damage DNA, proteins, and carbohydrate. These induce DNA strand breaks, as well as oxidation of purine and pyrimidine bases, and increase the occurrence of mutations(6). In addition, ROS can activate or inactivate proteins by oxidizing sulphhydryl groups and modifying amino acids(7,8).

In cells differ profoundly in their resistance to oxidant stress, depending on the balance between the prooxidants such as heavy metals and antioxidants in these cells. The cellular radical-scavenging systems include(9,10): molecules such as glutathione, glucose and albumin, Vitamins E, C, as well as minerals such as selenium and zinc etc, and enzymes such as glutathione peroxidase (GPX), catalase (CAT), superoxide Dismutase (SOD).

SOD is the major antioxidative enzyme(3). It was subsequently found to be a universal enzyme which exists in eukaryotes in three different metalloforms(12), manganese superoxide dismutase (Mn-SOD), intracellular superoxide dismutase (CuZn-SOD) and Extracellular superoxide dismutase (EC-SOD).
This enzyme has the function of disproportionating superoxide radicals by successive oxidation and reduction of the transition metal ion at its active site in a ping-pong type mechanism with remarkably high reaction rates (12), to oxygen and hydrogen peroxide.

Since elucidation of the biochemical mechanism involved in metastasis could provide a basis for the rational development of more effective anticancer therapies. Previously it has been shown that the oxidative stress is present in brain tumor (13). The aim of this study is to investigate the changes in the level of SOD in serum and tissue of patients with different brain tumors.

PATIENTS AND METHODS

Blood samples were collected from 33 healthy persons as a control group. Blood and brain tissue samples of different types and stages (30 serum samples, and 8 tissue samples) from patients with benign tumors and (32 serum samples, and 7 tissue samples) from patients with malignant brain tumor were collected from patients of 5-70 years. These samples were collected from Al-Gomla Al-Asabia hospital in Baghdad city.

Sample Preparation

Five milliliters of venous blood samples were collected in test tubes. The serum was separated immediately from the cells by centrifugation at 3000 x g for 5 min, meanwhile tissues samples were collected immediately after the operation in test tubes and Rinsed with normal saline.

The tumor tissue was taken out of saline and prepared for SOD activities measurement as the following procedure (14). The tumor tissue was washed with saline, to remove red blood cells and Minced using scissors, and homogenized with 10 volumes of acetate buffer (50 mM) pH 5.5 containing potassium bromide (0.3 M), using a manual pestle homogenizer for 5 min. The presence of KBr, which act as chaotropic salt, is necessary in the homogenization buffer. This salt was found to increase the yield of EC-SOD activity up to 2-3 fold, as suggest by marklund (14). The homogenate was placed on ice bath and sonicated at 10 Amplitude for 1 min at intervals of 15 sec. The homogenate was then incubated for 30 min at 4°C in order to complete the potassium bromide extraction. Finally the sample was centrifuged (8000 x g) for 30 min.

Enzyme activity assay

The activity of SOD enzyme in serum and supernatant homogenate was assayed by riboflavin/NBT method (15). The unit of SOD enzyme activity is defined as the amount of enzyme causing half the maximum inhibition of NBT reduction, while the enzyme specific activity was expressed as a unit of enzyme activity per mg of protein.

Determination of protein concentration

The protein concentration was determined using Lowry method for sera samples (16) and the modified Lowry method (17) for the supernatant homogenate.

RESULTS AND DISCUSSION

In order to evaluate the effect of this enzyme in patients with different brain...
tumors, its activity was measured in tissues and sera of these groups of patients and compared with that of benign and control groups respectively.

The level of sera SODs activity and specific activity, of control and of patients with different types of brain tumors is presented in Table (1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Size (n)</th>
<th>Activity Mean (U/mL)</th>
<th>Activity Standard deviation</th>
<th>Specific activity Mean (U/mg)</th>
<th>Specific activity Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>33</td>
<td>21.802</td>
<td>3.658</td>
<td>0.295</td>
<td>0.044</td>
</tr>
<tr>
<td>Benign</td>
<td>30</td>
<td>18.738</td>
<td>4.130</td>
<td>0.227</td>
<td>0.053</td>
</tr>
<tr>
<td>Malignant</td>
<td>32</td>
<td>17.008</td>
<td>3.423</td>
<td>0.193</td>
<td>0.046</td>
</tr>
</tbody>
</table>

As it is obvious from the results, the level of SOD activity in sera varied, and a significant decrease was noticed in this level between normal and benign group (P<0.01), whereas a highly significant decrease between normal and malignant group (P<0.001) was noticed. No significant decrease in sera SOD activity between benign and malignant group (P>0.05) was present. Specific activity gives obvious differences among these groups where highly significant differences of the enzyme specific activity were present in the normal sera in comparison to that of benign, and of malignant group (P<0.001).

A significant differences (P<0.01) were noticed between benign and malignant groups. The presence of SODs isoenzyme in plasma is in all probability the result of passive leakage from cells (14). It is possible that the low, but apparently regulated SOD activity noticed is a compromise between the need to protect the extracellular-fluid components and cell surfaces against (14). The decrease in sera SOD activity levels observed in patients with brain tumors may also be due to the decrease in the synthesis and release of SOD from the white blood cells since it is known that immune deficiency response occur upon the malignant transformation(18,19). Such deficiency appears, for example, when enhanced serum levels of ganglioside. For example lipid-bound sialic acids (LSA) are released by the tumor cells. LSA binds to the plasma membranes of the mononuclear cells and inhibits their functions. Such a behavior may be an important mechanism for immuno suppression in patients with malignant diseases(20).

The other reason, for the decrease in SOD activity, noticed in this study, may be due to progressive enzyme inactivation by its product H₂O₂(21,22).

The activity and specific activity of tissue samples of the above mentioned groups were compared and the results shown in Table (2).
Table (2): SOD activity and specific activity in tissue homogenate of patients with brain tumor

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Size (n)</th>
<th>Activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (U/mL)</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Benign</td>
<td>8</td>
<td>33.71</td>
<td>2.56</td>
</tr>
<tr>
<td>Malignant</td>
<td>7</td>
<td>31.00</td>
<td>3.52</td>
</tr>
</tbody>
</table>

The results that there is no significant variation in SOD activity and specific activity when these values were compared between benign and malignant groups (P > 0.05).

The results show that SOD activity is higher in the tissue homogenate than in the sera of the studied groups, the rest of the work in this study deals with the study of the enzyme in the sera samples rather than the tissue samples due to the difficulties in obtaining the tissue samples.

In order to check the variation in SOD specific activity according to the sex, the different groups that were used as subject of this study were divided into two subgroups: male and female groups.

In male sera, Figure (2a), highly significant decreases (P < 0.001) in SOD specific activity between normal and benign groups, normal and malignant groups, were noticed. While no significant decrease in this specific activity was noticed between benign and malignant male patient. In female, Figure (2b), SOD specific activity was found to significantly decrease (P<0.01) in benign group compared with that of normal, and in malignant group compared with that of patients with benign brain tumors. By comparison, a highly significant decrease (P<0.001) is enzyme specific activity in sera of patients with malignant tumors in comparison with that of normal cases were observed.

![Figure (2a): SOD enzyme specific activity in sera of control and male patient with different brain tumors](image-url)
The decrease in SOD activity in sera of patients harboring a malignant brain tumors, come in agreement with studies of Ikeda and Long(23), who have suggested that tumor cells in experimental brain tumors can produce superoxide radicals while free radical scavengers such as SOD and catalase in such tumor cells were impaired.

These results disagree with Alhabal(24), who reported an increase in SOD activity in the plasma of patients with breast cancer.

Decrease with no significant differences (P>0.05) were noticed in sera SOD specific activity among the female in comparison with that of male normal and malignant cases (Figure 3).
This result comes in agreement with what reported that the significant sex difference in SOD levels were observed in Chronic Myeloid Leukemia (CML) patients, where males showed higher levels of enzyme activity as compared to females. Also total antioxidant status (TAS) was lower in females than in males(25).

The decreases of SOD activity in female, in comparison with that of male, indicate the genetic difference between them in management of oxidative status(26). Estrogen is present in significant quantities in the plasma of female(27). This hormone thought to play a protective role against neurode-generation through a variety of mechanism including scavenging of ROS. Thus the role of estrogen as antioxidant may act as a substitution to SOD activity in female(28).

Malignant cases were divided into two groups as primary and secondary as illustrated in Table (3) and the SOD activity and specific activity were compared.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample Size (n)</th>
<th>Activity Mean (U/mL)</th>
<th>Standard deviation</th>
<th>Specific activity Mean (U/mg)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>30</td>
<td>18.738</td>
<td>4.130</td>
<td>0.227</td>
<td>0.053</td>
</tr>
<tr>
<td>Primary malignant</td>
<td>27</td>
<td>16.598</td>
<td>3.186</td>
<td>0.189</td>
<td>0.048</td>
</tr>
<tr>
<td>Secondary malignant</td>
<td>5</td>
<td>19.044</td>
<td>4.202</td>
<td>0.213</td>
<td>0.026</td>
</tr>
</tbody>
</table>

The results shows, a reduction in these parameters is present, when the comparison is made between benign and patients with primary brain tumor. This reduction is of low significant (P<0.05), and of significant values (P<0.01) in sera SOD activity and its specific activity respectively. When these values were compared between sera of patients with primary and secondary brain tumors, a significant rise (P<0.05) was noticed. The increases in SOD activity and specific activity were recorded in the sera of patients with secondary tumor in comparison to those with primary brain tumors. This may be due to a metastatic cell, which penetrates the extracellular matrix (ECM) that surrounds the tumor, and then travels through the
tissue till it reaches a blood vessel or a lymphatic. The tumor cell then attaches to the blood vessel wall, where the invasive tumor cell secretes certain proteolytic enzymes that degrade the basement membrane (BM). Several proteolytic enzymes have been included: incriminated collagenases, heparanase, cathepsin B, plasmin. Many metastatic cells produce heparanase that degrades heparan SO₄, and the predominant proteoglycan of the basement membrane(29). In the c-terminal end of human EC-SOD C, there is a cluster of positively charged amino acid residues(30), with a high affinity for certain glycosaminoglycans such as heparin and heparan sulphate(31). Thus, the degrade heparan sulphate of BM leads to release EC-SOD C into the circulating blood. At a distant site, the tumor cell again re-attaches to the blood vessel wall and repeats the process, traveling as much as two or three cell diameters into the invaded tissue before it settles down and begins to form a new tumor(29). Therefore, the increase in SOD specific activity in secondary malignant cases, in comparison with primary malignant cases, is due to the release of EC-SOD C from BM into the circulating blood in consequence to metastasis process.

The results in Table (1) and Table (2) show that SOD activity is higher in tissue than in sera samples, and this is expected, since this enzyme is an intracellular one that functions mainly within the cell(32). Also, this results agree with what was reported about the extracellular space, which has a little enzymatic protection against oxy-radicals, and about the extracellular space may be an especially likely site in the body for oxy-radical-mediated damage(14).

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