Study of the changes of vascular endothelial growth factor (VEGF), antioxidant capacity and oxidative stress in the sera of Iraqi patients with ischemic stroke

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Abstract

Background: Ischemic stroke is death of brain tissue due to interruption of blood flow to a region of the brain, caused by occlusion of a carotid or vertebral artery or, less likely, a cerebral vein. It accounts for 85% of all stroke cases. Ischemic stroke is rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer, or leading to death.

Aim of the study: To find out the relation between vascular endothelial growth factor, antioxidant capacity and oxidative stress in the patients with acute ischemic stroke cases.

Patients and Methods: Our study was conducted on (60) patients with acute ischemic stroke and (30) apparently healthy subjects were taken as control group. The sera obtained from the blood of patients and healthy subjects were used to measure the concentrations of vascular endothelial growth factor (VEGF), total antioxidant capacity (TAC) and malondialdehyde (MDA). Serum VEGF concentration was determined by using enzyme-linked immunosorbent assay (ELISA). Total antioxidant capacity and malondialdehyde (MDA) were determined by using colorimetric method.

Results: In comparison with the control group, the patients with acute ischemic stroke show a significant increase in VEGF (P < 0.01) and MDA (P < 0.01) concentrations, and a significant decrease in TAC concentration (P < 0.01). Also we found a significant elevation of VEGF (P < 0.05) in hypertensive patients with ischemic stroke compared to normotensive patients. On the other hand, there was a significant decrease (P < 0.05) of TAC concentration in smoker patients compared to non smoker ones against a significant increase (P < 0.05) of MDA concentration of smoker patients compared to non smoker patients. Also MDA level found to be increase significantly (P < 0.05) in ischemic stroke patient who were also diabetic in compared to non diabetic patients.

Significant positive correlation also observed between VEGF and MDA concentrations (r=0.374, P<0.05) in patient group.

Conclusion: Our results suggest a functional interplay among oxidative stress, antioxidants, and VEGF in patients with ischemic stroke.

Keywords: Ischemic stroke, Vascular endothelial growth factor, Total antioxidant capacity, Malondialdehyde.
Introduction

Stroke or what is known medically as cerebrovascular accident (CVA) is the third most common cause of death in industrialized countries, following coronary heart disease and cancer. It is also the most prevalent neurologic disorder in terms of morbidity and mortality (Yildirim A, et al. 2007).

The world health organization (WHO) defines stroke as "rapidly developing clinical signs of focal ("or global") disturbance of cerebral function, with symptoms lasting 24 hours or longer, or leading to death, with no apparent cause other than of vascular origin". If the symptoms lasting less than 24 hours, the event is defined as a transient ischemic attack (TIA) (World Health Organization, 2006).

Brain ischemia initiates a complex cascade of metabolic events, several of which involve the generation of nitrogen and oxygen free radicals. These free radicals and related reactive chemical species mediate much of the damage that occurs after transient brain ischemia, and in the penumbral region of infarcts caused by permanent ischemia (Seth L. 1999).

VEGF is one of the most potent and specific angiogenic cytokines. It is a 23 to 45 kDa heparin-binding glycoprotein that exerts multiple effects on tumors, including stimulating the formation of new blood and lymphatics vessels and increasing vascular permeability (Folkman J. 1995). In the brain, VEGF is abundantly produced from neurons and vascular cells and acts on themselves, thereby playing an important role in mediating neuronal survival and angiogenesis (Sun Y, et al. 2003).

Patients and Methods

This study was performed at the laboratory of Biochemistry Department, College of Medicine and DNA research centre, College of science, University of Babylon.

The patients group who subjected to this study were (60) persons in the age group ranging from 44 - 86 years, the mean ± standard deviation (SD) was (62.3 ± 11.29) years. This group comprises of (60%) males (61.8 ± 11.4 years), and (40%) females (63.1 ± 11.3 years). The diagnosis and the type of stroke were confirmed by CT- scanning or MRI- imaging techniques.

Thirty apparently healthy individuals were taken as a control group in the age group ranging from 43 - 82 years, the mean ± standard deviation (SD) of age was...
This group comprises of (63%) males (60.4 ± 10.2 years), and (37%) females (64.5 ± 11.6 years).

The age and sex of this group were matched to age and sex of patient group, where statistical analysis showed non significant differences in the age and sex between patient and control groups (p > 0.05).

The present study revealed that 70% of the patients had hypertension, 42% were smokers, 30% had an ischemic heart disease, 25% of the patients were experienced a previous stroke and 25% were diabetics, the main risk factors of patient group and their order were summarized in table (1).

All statistical analysis were performed by using SPSS 17 version. Data were expressed as (mean ± SD). The normality of the distribution of all variables was assessed by the student’s ANOVA test and Pearson correlation analyses that have been used to determine the significant difference between the groups.

Results and Discussion

The results of the present study were summarized in Table (2). In the present study, plasma VEGF values were significantly higher in stroke patient group than control group (P < 0.01). Upon hypoxia and ischemia, VEGF and its receptors are rapidly induced within hours on neurons and glial cells (Sun Y.et al. 2003). In the acute phase of ischemic stroke, VEGF exerts multiple actions in the peri-infarct region that include the promotion of neuronal survival (Wang Y.et al. 2005), angiogenesis (Zhang ZG.et al. 2000), and neural progenitor cell proliferation, migration and differentiation (Wang Y-Q.et al. 2005). Hypoxia-inducible factors are well-characterized transcription factors that upregulate VEGF (Marti HJ.et al. 2000).

Also a significant elevation of VEGF (P < 0.05) in hypertensive patients with ischemic stroke compared to normotensive patients, as shown in table (3). Since the control group had no hypertensive subjects, a comparison was made of the mean level of VEGF between normotensive patients and control group, resulting in a significant difference (P < 0.05), this will eliminate the possibility that increased level of VEGF is due to hypertension alone not due to ischemic stroke itself. Previous study has demonstrated that VEGF is increased in hypertensive patients and decreased after control of blood pressure (Wei-Chuan Tsai, et al. 2005).

There are several proposed mechanisms responsible for the elevation of VEGF in vascular diseases. First, in response to vascular damage, a wide array of growth factors, cytokines and other molecules are released, stimulating angiogenesis via VEGF, which is essential for the repair process. Another possible mechanism is that elevation of VEGF may simply reflect endothelial cell damage apparent in hypertension (Felmeden, D. C.,et al. 2003).

Also the present study revealed that plasma concentration of (TAC) was decreased significantly in patients with ischemic stroke compared to control group (P < 0.001). Unstable free radical species attack cellular components causing damage to lipids, proteins, and DNA (Droge W. 2001). Living organisms have developed complex antioxidant system to counteract (ROS) and to reduce their damage (Pamela C. 2005). The sum of endogenous and food-derived antioxidants represents the total antioxidant capacity (i.e. TAC) of the system.

Significant decrease in (TAC) in patients with ischemic stroke compared to control group observed in this study may be attributed to oxidative stress, which causing free radical formation resulting in tissue damage, and imbalance between the production and detoxification of (ROS) results in oxidative stress (YEH.et al. 2005). This study suggests a significant decrease in TAC level in sera of smoking patients with ischemic stroke than non smoking patients (P < 0.05), as shown in table (3).
Since the control group had no smoking subjects, a comparison was made of the mean level of TAC between non-smoker patients and control group, resulting in a significant difference (P < 0.05), this will eliminate the possibility that decreased level of TAC is due to smoking alone not due to ischemic stroke itself.

The significantly lower TAC levels observed in smokers in this study, may be due to the inflammatory state associated normally with smoking may influence the optimal antioxidant concentrations for physiological protection. Thus, in smokers, in whom evidence exists of a heightened inflammatory status, lower concentrations of plasma antioxidants may be an adaptation to this state (Jens Lykkesfeldt, et al. 2004).

In the same context, plasma concentration of (MDA) was increased significantly in patients with ischemic stroke compared to control group (P 0.01). Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation (Basu AK, Marnett LJ.1983). Rise in MDA could be due to increased generation of reactive oxygen species (ROS) that may result from excessive oxidative damage generated in stroke patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar reports of higher MDA levels in stroke patients were observed by (Cano CP et al, 2003), (Beg M et al, 2005), (Bir LS et al, 2006), (Yildirim A et al, 2007), (Sarker PD et al, 2009). (Nattheer H et al. 2009).

This study also revealed that smoking and diabetes mellitus can affect the level of MDA in patients with ischemic stroke, as shown in table (3). Since the control group had no smoking subjects, a comparison was made of the mean level of MDA between non-smoker patients and control group, resulting in a significant difference (P < 0.05), this will eliminate the possibility that elevated level of MDA is related to smoking alone not to ischemic stroke itself. Also similar comparison was made of mean level of MDA between non-diabetic patients and control group, a significant difference (P < 0.05) was noticed, that’s means that elevated level of MDA in patients is related to ischemic stroke.

The significantly higher MDA levels observed in smokers in this study, may be due to one or more of the following reasons: First of all, smokers are prone to oxidation from inhalation of large numbers of gas-phase and other radicals giving rise to increased oxidative damage (Hanta I, et al. 2006). Second, depletion of plasma antioxidants, otherwise protecting against oxidative damage such as lipid peroxidation, that has consistently been observed among smokers (Jens Lykkesfeldt et al. 2004). This result is of good agreement with some previous studies (Block G, et al. 2002), (Goraca A and Skibska B. 2005).

Increased oxidative stress in diabetes has been attributed to enhanced flux of reactive oxygen species generated by auto-oxidative glycation reactions that results in a variety of changes such as glycation, glyco-oxidation, and lipid peroxidation, and ultimately leads to the formation of advanced glycation end products (Schmidt A, et al. 1994).

This study also shows a positive correlation between VEGF level and MDA level in patient group (r = 0.374, P < 0.05).

The process of angiogenesis requires a change in the local equilibrium between pro angiogenic and anti angiogenic factors (Rak J, et al. 1995). Oxidative stress, after causing nonlethal injury to cells, may initiate a signal transduction cascade leading to tissue repair and angiogenesis (Maulik N, Das DK. 2002).
Table (1) The order of risk factors among patients group.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>No. of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>42</td>
<td>70 %</td>
</tr>
<tr>
<td>Smoking</td>
<td>25</td>
<td>42 %</td>
</tr>
<tr>
<td>Diabetes</td>
<td>15</td>
<td>25 %</td>
</tr>
</tbody>
</table>

Table (2) Biochemical parameters of patients with ischemic stroke and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>VEGF (pg/ml) Mean ± SD*</th>
<th>TAC (mmolar) Mean ± SD</th>
<th>MDA (µmolar) Mean ± SD</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>108.6 ± 16.25</td>
<td>1.49 ± 0.22</td>
<td>2.5 ± 0.48</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Patient</td>
<td>367.99 ± 70.99</td>
<td>1.18 ± 0.23</td>
<td>5.9 ± 0.87</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

* SD = standard deviation.
** P value < 0.01 considered highly significant.

Table (3) Effect of stroke risk factors on MDA level

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Subclass</th>
<th>VEGF (pg/ml) Mean ± SD</th>
<th>TAC (mmolar) Mean ± SD</th>
<th>MDA (µmolar) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Hypertensive</td>
<td>394 ± 79.3*</td>
<td>1.16 ± 0.22</td>
<td>6 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>Normotensive</td>
<td>342.1 ± 35.2</td>
<td>1.22 ± 0.22</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td>Smoking</td>
<td>Smokers</td>
<td>367.4 ± 66.7</td>
<td>1.04 ± 0.19*</td>
<td>7.1 ± 0.8*</td>
</tr>
<tr>
<td></td>
<td>Non smokers</td>
<td>368.4 ± 74.8</td>
<td>1.32 ± 0.24</td>
<td>4.7 ± 0.97</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Diabetic</td>
<td>387.6 ± 87.7</td>
<td>1.13 ± 0.23</td>
<td>7 ± 0.83*</td>
</tr>
<tr>
<td></td>
<td>Non diabetic</td>
<td>362.6 ± 65.7</td>
<td>1.23 ± 0.21</td>
<td>4.8 ± 0.88</td>
</tr>
</tbody>
</table>

* Significant difference (P < 0.05).

References

Al-Rawi NH, Jaber FA, Atiyah KM. (2009); Assessment of salivary and serum oxidative stress and antioxidants as plausible parameters in prediction of ischemic stroke among Iraqi Samples. The Internet Journal of Third World Medicine7(2).


