Oxidative stress has been found to be involved in both stroke and renal dysfunction. In the present study, the relationship of oxidative stress and renal dysfunction after acute stroke was investigated.

Our study was conducted on seventy four patients with acute stroke and forty one apparently healthy subjects were taken as control group. Patients group was divided into two subgroups, patients with ischemic stroke and patients with hemorrhagic stroke. The sera obtained from the blood of patients and healthy subjects were used to measure the concentrations of malondialdehyde (MDA), glutathione (GSH), creatinine (SCr), and blood urea nitrogen (BUN), and the specific activity of glutathione peroxidase and catalase. Estimated glomerular filtration rate (eGFR) was also calculated.

The results of this study showed that MDA, SCr, and BUN concentrations were found to be significantly higher, and GSH concentration, GPX and CAT activities, and eGFR levels were found to be significantly lower in acute stroke patients than control group (P < 0.001). Patients with ischemic stroke have significant increase in MDA and SCr concentrations (P< 0.01, P< 0.05, respectively), and significant decrease in GSH concentration, GPX and CAT activities, and eGFR levels (P< 0.05, P< 0.05, P< 0.01, P< 0.01, respectively) than patients with hemorrhagic stroke.

Our results also showed the presence of highly significant positive correlation between MDA concentration and BUN and SCr concentrations, and highly significant negative correlation between MDA concentration and eGFR levels. Inversely there was highly significant negative correlation between measured antioxidants levels and BUN and SCr levels, and highly significant positive correlation between measured antioxidants and eGFR levels.

According to the results of our study we found that acute stroke is associated with elevated oxidative stress, kidney function is significantly compromised in patients with acute stroke, acute kidney injury (AKI) is a common complication after acute stroke, and there was highly significant correlation between oxidative stress in patient with acute stroke and decline in kidney function.

The kidney function in patient with acute stroke may be directly or indirectly affected by the level of oxidative stress developed during the acute phase of disease and there was a cerebro-renal connection, which is disease that affects brain may also affect kidney and vice versa.
Oxidative stress in patients with acute stroke…. 
Ali H. Husein

Introduction

Stroke or 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 term of morbidity and mortality, \(^1\) and is the primary cause of adult disability in developed countries.\(^2\)

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).\(^3\)

The two major mechanisms causing brain damage in stroke are ischemia and hemorrhage.\(^4\) 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 damage that occurs after transient brain ischemia, and in the penumbral region of infarcts caused by permanent ischemia.\(^5\)

Free radicals and reactive oxygen species (ROS) have been associated with the etiology and/or progression of a number of diseases, aging, and organ failure. The generation of highly ROS is an integral feature of normal cellular function like mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation, and fertilisation.\(^6\) Their production however,
Oxidative stress multiplies several folds during pathological conditions.\(^7\) Oxidant-induced alterations of proteins, membranes, DNA, and basement membranes leads to cell and organ dysfunctions.\(^8\) Sies,\(^9\) defines oxidative stress as “a disturbance in the pro-oxidant–antioxidant balance in favour of the former, leading to potential damage”. Oxidative stress can thus occur when the production of free radicals increases, scavenging of free radicals or repair of oxidatively modified macromolecules decreases, or both. This imbalance results in a build-up of oxidatively modified molecules that can cause cellular dysfunction and, for postmitotic cells such as neurons, cell death.\(^10\)

There is an increasing amount of experimental evidence that oxidative stress is a causal, or at least an ancillary, factor in the neuropathology of several adult neurodegenerative disorders, as well as in stroke, trauma, and seizures.\(^11\) Also the relationship between oxidative stress and renal failure had been found in many studies.\(^12-14\)

There are conflicting published data about the association of renal dysfunction with cerebrovascular diseases. In some studies, renal dysfunction has been found to be a risk factor for cerebrovascular diseases.\(^15-17\) in other studies it is consider as a prognostic factor after stroke.\(^18,19\) In present work the association between oxidants/antioxidants, acute stroke and renal function have been studied.

### Materials and Methods

#### Materials

This study was performed at the laboratory of Biochemistry Department, College of Medicine, University of Babylon. The collection of samples was conducted during the period from 1\(^{st}\) of December 2010 till 30\(^{th}\) of June 2011. The patients group whom subjected to this study were Seventy four subjects in the age group ranging from 33-100 years, the mean ± standard deviation (SD) was (66.62±12.99 years). All of those patients admitted to Merjan Teaching Hospital in Hilla city with clinical symptoms of acute stroke. The diagnosis and the type of stroke were confirmed by CT-scanning or MRI-imaging techniques. The patients group subdivided into two groups according to type of stroke:

1. Ischemic stroke group: which comprises of 59 patients, 28 male and 31 female
2. Hemorrhagic stroke group: which comprises of 15 patients, 10 male and 5 female

All patients were in the acute state of disease. The patients with a history of renal diseases and patients whose clinical symptoms and imaging not confirmed stroke, or only diagnosed as having TIA have been excluded from this study. Age and sex matched forty one apparently healthy individuals, taken as control group. Their age ranging from 36 – 90 years with mean ± SD of 66.14 ± 14.15 years.

Blood samples, about 5 ml were collected from patients and healthy volunteers by vein puncture and pushed slowly into plain disposable tubes without any additives. Blood was allowed to clot at 37°C for 10-15 minutes, and then centrifuged at 3000 rpm for approximately 10-15 minutes then the sera were obtained and stored at -20°C until analysis. The sera from patients and control group are used for the measurements of the following parameters: Total protein concentration (TP), creatinine concentration (SCr), BUN concentration, GSH concentration, CAT activity, GPX activity, and MDA concentration.
Oxidative stress in patients with acute stroke….

Ali H. Husein

Methods

Serum total protein concentration measured by total protein kit provided from Biolabo Company (France). BUN measured by urea kit provided from Human Company (German). SCr measured by creatinine kit provided from Spinreact Company (Spain). The glomerular filtration rate was estimated by means of (four-variable) modification of diet in renal disease (MDRD) equation, which estimates GFR using four variables: serum creatinine, age, race, and gender. Serum MDA concentration are determined by Carl A. Burtis and Edward R. Ashwood procedure. Serum GSH concentration was measured by Ellman's method. Catalase (CAT) activity was determined by the measurement of the decrease in the absorbance due to hydrogen peroxide H$_2$O$_2$ consumption as described by Aebi H. Glutathione peroxidase (GPx) was assayed according to the procedure of Rotruck et. al with some modification.

Statistical analysis

All statistical analyses were performed by using SPSS 15 software for Windows. Data were expressed as mean ± SD. The normality of the distribution of all variables was assessed by the Kolmogorov-Smirnov test. Student's t test and Pearson correlation analyses were used for normally distributed variables. Mann-Whitney U test and Spearman rank correlation test were used for nonparametric variables.

Results

Malondialdehyde

Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation. In our work the mean ± SD of MDA concentration in stroke patients and control groups was 7.40 ± 3.03 and 2.39 ± 0.72 µmol/l, respectively. Statistical analysis shows that there was a significant increase in MDA concentration in stroke patients compared to healthy control group (p<0.001).

The results of our study also show that there was a significant difference in the mean of MDA concentration between patients with hemorrhagic and ischemic stroke (p<0.01). Ischemic stroke patients have increased MDA concentration compared to hemorrhagic stroke patients, mean ± SD was 7.87± 2.94 and 5.53± 2.69 respectively, as shown in table (1).

Table 1: The distribution of MDA among stroke patients and control group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>No.</th>
<th>mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/l)</td>
<td>Patients</td>
<td>74</td>
<td>7.40± 3.03</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>41</td>
<td>2.93± 0.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ischemic</td>
<td>59</td>
<td>7.87± 2.94</td>
<td>P&lt;0.01**</td>
</tr>
<tr>
<td></td>
<td>Hemorrhagic</td>
<td>15</td>
<td>5.53± 2.69</td>
<td></td>
</tr>
</tbody>
</table>

**: the difference is significance at p=0.01.

Glutathione (GSH), glutathione peroxidase (GPX), catalase (CAT)

Serum reduced GSH was measured as enzymatic antioxidants. The mean ± SD of these parameters in various groups was shown in table (2). The results in the preceding table show that acute stroke patients have highly significant decrease in GSH
concentrations, and GPX and CAT activities compared to control group (P< 0.001), also patients with ischemic stroke have a significantly low GSH concentrations (P< 0.05), and highly significant decrease in GPX and CAT activities (P<0.01) compared to patients with hemorrhagic stroke. Also the results show that there was no significant difference in GSH, GPX and CAT concentrations between males and females with acute stroke.

**Table 2: GSH, GPX and CAT levels in patients and control group.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (µmol/l)</th>
<th>P value</th>
<th>GPX (U/gm protein)</th>
<th>P value</th>
<th>CAT (U/mg protein)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.48 ± 2.21</td>
<td>P&lt;0.01**</td>
<td>0.49 ± 0.19</td>
<td>P&lt;0.01</td>
<td>0.21 ± 0.09</td>
<td>P&lt; 0.01</td>
</tr>
<tr>
<td>Ischemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>4.16 ± 2.11</td>
<td>P&lt;0.05*</td>
<td>0.46 ± 0.18</td>
<td>P&lt;0.01</td>
<td>0.19 ± 0.09</td>
<td>P&lt; 0.05</td>
</tr>
<tr>
<td>Male</td>
<td>4.87 ± 2.33</td>
<td>NS</td>
<td>0.53 ± 0.20</td>
<td>NS</td>
<td>0.22 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>4.06 ± 2.01</td>
<td></td>
<td>0.46 ± 0.18</td>
<td></td>
<td>0.19 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

*: the difference is significant at p=0.05.
**: the difference is significant at p= 0.01.
a: Nonsignificant difference.

**Blood urea nitrogen (BUN), serum creatinine (SCr), estimated glomerular filtration rate (eGFR)**
The Results of our study have been shown that there were highly significant increase in BUN and SCr concentrations in patients group compared to control group (p< 0.001), on the other hand patients group had significantly lowered eGFR levels compared to control group (P< 0.001), as shown in table (3):

**Table 3: BUN, SCr, and eGFR levels in patients and control groups.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN(mg/dl)</td>
<td>Patients</td>
<td>37.45 ± 14.25</td>
<td>P&lt; 0.001**</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>17.95 ± 6.46</td>
<td></td>
</tr>
<tr>
<td>SCr(mg/dl)</td>
<td>Patients</td>
<td>1.70 ± 0.71</td>
<td>P&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.85 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>eGFR(ml/min/1.73m²)</td>
<td>Patients</td>
<td>42.19 ± 26.61</td>
<td>P&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>95.85 ± 21.25</td>
<td></td>
</tr>
</tbody>
</table>

Patients with ischemic or hemorrhagic stroke had non significant differences (p> 0.05) in BUN concentration, while there were highly significant increase in SCr concentration and highly significant decrease in eGFR levels in patients with ischemic stroke compared to patients with hemorrhagic stroke (p< 0.01), as shown in table (4):

**Correlation between oxidants/antioxidants and BUN, SCr, eGFR.**
The results of our study show highly significant positive correlation between MDA concentration and BUN and SCr levels. Our results also show the presence of highly significant negative correlation between MDA and eGFR. The results also show highly significant negative correlation
between antioxidants (GSH, GPX, and CAT) and BUN and SCr concentrations, and highly significant positive correlation between antioxidants and eGFR levels, as shown in table (5):

Table 4: BUN, SCr, and eGFR levels in ischemic and hemorrhagic stroke patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dl)</td>
<td>Ischemic</td>
<td>38.74 ± 14.55</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Hemorrhag</td>
<td>32.40 ± 12.17</td>
<td></td>
</tr>
<tr>
<td>SCr (mg/dl)</td>
<td>Ischemic</td>
<td>1.78 ± 0.71</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Hemorrhag</td>
<td>1.36 ± 0.63</td>
<td></td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>Ischemic</td>
<td>37.56 ± 23.15</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Hemorrhag</td>
<td>60.41 ± 32.03</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Correlation between oxidants/antioxidants and kidney function tests.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BUN</th>
<th>SCr</th>
<th>eGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r-value</td>
<td>p-value</td>
<td>r-value</td>
</tr>
<tr>
<td>GSH</td>
<td>−0.468</td>
<td>P &lt; 0.01</td>
<td>−0.508</td>
</tr>
<tr>
<td>GPX</td>
<td>−0.547</td>
<td>P &lt; 0.01</td>
<td>−0.604</td>
</tr>
<tr>
<td>CAT</td>
<td>−0.348</td>
<td>P &lt; 0.01</td>
<td>−0.568</td>
</tr>
<tr>
<td>MDA</td>
<td>0.515</td>
<td>P &lt; 0.01</td>
<td>0.777</td>
</tr>
</tbody>
</table>

Acute kidney injury and stroke

The Acute Kidney Injury Network (AKIN) defines AKI as an abrupt (within 48h) reduction in kidney function characterized by an absolute increase in serum creatinine of either ≥0.3 mg/dl (≥25 µmol/L) or a percentage increase of ≥50% or a reduction in urine output (documented oliguria <0.5 ml/kg per h for >6 h). By using means of BUN and SCr of healthy control group (which are 17.95 and 0.85 mg/dl, respectively) as a baseline levels of these parameters in patient group, we found that 55 patients (74.32%) met AKIN criteria and defined as having AKI. Also We found that AKI was more prevalent in patients with ischemic stroke compared to patients with hemorrhagic stroke, where from 59 patients having ischemic stroke, 48 (81.40%) defined as having AKI , where as of 15 patients With hemorrhagic stroke, only 7 (46.70%) defined as having AKI.

Discussion

Rise in MDA concentration 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.,(27) Beg M et al.,(28) Bir LS et al.,(29) Yildirim A et al.,(1) Sarker PD et al.,(30) Natheer H et al.(31) Ischemic stroke patients have increased MDA concentration compared to hemorrhagic stroke patients, and this may be due to the aetiology of ischemic and hemorrhagic stroke. Most ischemic stroke are due to occlusion of cerebral arteries or veins by thrombi or emboli.(4) Some of the neurons served by the occluded vessel die from lack of oxygen and nutrients. This results in cerebral infarction, in which tissue injury triggers an inflammatory response and disrupts metabolism and leads to changes in ionic transport, localized acidosis, and free radical formation.(32) Hemorrhagic stroke occur mainly due to rupture of a blood vessel, hemorrhage into the brain occurs, resulting in edema, compression of the brain contents, or spasm of the adjacent blood vessels. (33)
GSH is a free radical scavenger and a proton donor for GPX, which is known to have a neuroprotective role. It is reported that depletion of total GSH and a decreasing GSH to GSSG ratio are markers for oxidative stress in an ischemic brain. In the present study, the level of serum GSH in acute stroke patients were significantly lower compared to those in the controls, and this agreed with the result of Yildirim A et al study. On the other hand, a study of Zimmermann C et al stated that GSH and GPX were elevated in the first hours and days after the acute stroke, and a study of Gariballa SE et al stated that Baseline glutathione concentrations were non-significantly lowest in ischaemic stroke patients compared with controls.

Glutathione peroxidase (GPX) is one of the primary antioxidant enzymes that scavenge hydrogen peroxide and organic hydroperoxides with glutathione as the hydrogen donor during normal metabolism or following oxidative insults. We found a decline in the activity of serum GPX in the stroke patients as compared to the healthy controls, and this goes with the result of Jawalekar et al study. Catalase is a common enzyme found in nearly all living organisms, which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. In our study we found a highly significant decreased in catalase activity in acute stroke patients compared to healthy control subjects, and this concurrent with the results of De la Torre MR et al. The decreased in the levels of antioxidants in stroke patients compared to healthy control group was indicated a depletion in these antioxidants as a result of a greater extent of oxygen free radical scavenging action of these antioxidants, and this confirmed by high levels of lipid peroxidation product (MDA) in patients group compared to control group which indicate a greater degree of free radical generation. Once generated, free radicals can react with all cellular macromolecules, including proteins, and protein oxidation, particularly of enzymes, can lead to impairment of their function, and this leads to a greater degree of oxidative stress and oxidative damage.

Our study also shows a significant decline in GSH, GPX and CAT in ischemic stroke compared to hemorrhagic stroke, and a decrease in tissue antioxidant levels in the brain during ischemia have been reported as indirect evidence of oxidative stress. In our study we found that AKI is a common complication after acute stroke. Approximately three quarter of our patients developed AKI in the first day after the ictus. Because acute stroke patients included in this study were lacking any kidney problems before this time, renal dysfunctions that appeared in acute stroke patients (especially patients with ischemic stroke) was therefore considered to be an acute kidney injury (AKI) and occur as a complication of stroke events. The high incidence of AKI in our population can be explained by the increased age (mean age 66.62 ± 12.99 years), low baseline GFR, and the use of a high-sensitivity definition for the detection of AKI. Undiscovered preexisting renal dysfunction may be a major contributor to the occurrence of AKI.

One possibility for the development of AKI after stroke is an embolic event (blood clot moving from one place to another) that may have affected kidney function, where Most ischemic strokes are due to cerebral emboli which arise from the heart (cardioembolic stroke).
If the blood clot was thrown from the brain and moved to one or both of the kidneys then the kidneys could have had "strokes" too - thereby reducing kidney function. An alternative possibility for the development of AKI after stroke is that disturbing in the treatment of hypertension during the acute phase of stroke may leads to adverse effects on the kidneys, where both low and high blood pressures during the acute phase of stroke are associated independently with a poor outcome.

In our study as with many studies reported that oxidative stress increased dramatically in acute stroke patients especially ischemic stroke, where oxidative stress marker (MDA) significantly increased in ischemic stroke compared to hemorrhagic stroke, moreover kidney function in patients with ischemic stroke was found to be more worsened compared to patients with hemorrhagic stroke. All of these findings as well as the strong correlation between oxidants/antioxidants and kidney function tests, let us to hypothesize that oxidative stress which increased as a result of stroke events, or as a result of risk factors preceding the stroke may play a causative role, or contribute to the pathogenesis of stroke-induced AKI. However the mechanism of beyond this is not clear and may be related to free radicals modifications of lipid, proteins and DNA in the kidney that causes disruption of its functions.

Conclusions

From the results of our study we can conclude that acute stroke is associated with high degree of oxidative stress, and oxidative damage and these changes in the redox state of the body may affect kidney function directly or indirectly leading to various degrees of AKI after stroke. Therefore patients with acute stroke may need antioxidants treatment with extensive monitoring of kidney function.

References

11. Coyle JT, Puttfarken P. Oxidative Stress, Glutamate, and Neurodegenerative Disorders Science (1993); 262 (5134); 689-695.
Oxidative stress in patients with acute stroke.


31. Al-Rawi NH, Jaber FA, Atiyah KM. 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 Medicine (2009); 7(2).


45. Tunold Lund CG. Cerebral Emboli and Ischemic Brain Injury. Doctoral Thesis Faculty of Medicine, University of Oslo, Department of Neurology And The Interventional Centre (2006): PP. 1–56.