Biomarkers of Oxidative Stress in Major Depressive Disorder

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Abstract

BACKGROUND: Many data support that oxidative stress and inflammation represent a pathway to pathology in a number of depressed patients. Therefore, investigating this pathway presents an area for developing potential therapeutic strategies for depression.

AIM: This study compares the serum level of malondialdehyde (MDA), superoxide dismutase (SOD), and nuclear factor erythroid 2-related factor 2 (Nrf2) in depressed and non-depressed subjects and correlate between their levels with severity of disorder, socio-demographic characteristics, previous hospitalization, and number of episodes.

METHODS: A total of 60 patients diagnosed with major depressive disorder (MDD) from the Psychiatric Outpatient Clinic in Al-Zahra University Hospital, Cairo, Egypt, during the period from July 1, 2018, to December 31, 2018. A cross-matched control group of 60 subjects was selected from employees working in the hospital, complete psychiatric history, Hamilton Depression rating scale was done and serum levels of MDA, Nrf2, and SOD were estimated.

RESULTS: No statistical difference between patient and control group was observed regarding age, sex, education, and marital status. The number of patients with mild depression was 28 (46.7%), moderate depression 18 (30%), and severe depression 14 (23.3%). The mean duration of illness in years was 6.13, and mean number of episodes was 3.66. MDA level was significantly elevated in the patient group than the control one. Meanwhile, SOD and Nrf2 were significantly lower in the patient group than the control. There was significant relationship between duration of illness and number of episodes and MDA, Nrf2, and SOD levels.

CONCLUSION: Our results indicate that oxidative stress can contribute to the occurrence of MDD.

Introduction

Depression is a state of low mood and demotivation affecting individual’s feelings, cognition, and behaviors. It is the most common psychiatric disorder and is considered a high-risk factor of suicide [1].

Researchers suggest that depression does not stem from simply too much or too little brain chemicals. Many data support the hypothesis that inflammation represents a pathway to pathology in a number of depressed patients. Therefore, investigating this pathway presents an area for developing potential therapeutic strategies for depression [2].

According to the WHO; more than 300 million people suffer from depression. The WHO identified strong relations between depression and other disorders. It increases the risk of substance use disorders, diabetes mellitus, and heart disease [3]. At worst, it leads to suicide which claims 800,000 of lives every year [4]. Depression induces global disability (7.5 of all years lived with disability in 2015). Major depression is projected to become the second leading cause of disability all over the world [3].

A growing body of evidence supports the relation between depression and inflammation [5]. Studies showed more cytokines production in medicated depressed or drug free patients than controls [6]. Oxidative stress plays a role in neuronal cell death directly or through an inflammatory process. There is an increasing evidence of excess level of reactive oxygen species (ROS) and inflammatory biomarkers in depressed patients with activation of stress kinases promoting further oxidative stress and neuroinflammation with subsequent cell death. Oxidative stress leads to oxidized lipids, proteins, and nucleic acids as result of greater ROS [7] targeting fatty acids, monoamines of the brain. Monoamine oxidation produces superoxide anions; therefore, a critical balance is required between free radicals and antioxidants mechanisms.

Oxidative stress damage is evidenced by an increased level of malondialdehyde (MDA), polyunsaturated fatty acid peroxidation by product [8]. In the same context, nuclear factor erythroid 2-related factor 2 (Nrf2) is the master regulator of inducible antioxidant responses [9]. The activation of Nrf2 plays a key role in inflammation [10] through the reduction of ROS production.
and the macrophage M1 phenotype [11], regulating antioxidant response element (ARE) gene expression The Keap1 (Kelch-like ECH-associated protein 1), and the recruitment of inflammatory cells. During oxidative stress, free Nrf2 translocates to the nucleus and binds to ARE genes such as hemeoxygenase-1 inhibiting the NF-KB pathway which leads to downregulation of pro-inflammatory cytokines [12]. In inflammatory disorders and neurodegenerative diseases, Nrf2 stimulator has become an important therapeutic strategy [13], [14], [15]. Hence, the discovery of new Nrf2 stimulator for clinical study is an essential target in drug discovery.

Nrf2 has a crucial role in inflammation which is implicated in depression. Its deletion in mice results in reduced dopamine and serotonin in prefrontal cortex [16]. Furthermore, in a chronic stress paradigm, Nrf2 has a mechanism responsible for antidepressant response [17]. A study by Martín-Hernández (2018) [18] was done on postmortem brains of depressed patients showed decreased expression of Nrf2 in the dorsolateral prefrontal cortex. Furthermore, superoxide dismutase (SOD) is an enzyme that converts superoxide anion radicals to molecular oxygen and hydrogen peroxide controlling ROS [19].

**Aim**

The aim of the study was to compare the serum levels of MDA, Nrf2, and SOD in depressed and non-depressed subjects and to correlate between their levels with the severity of disorder, socio-demographic characteristics, previous hospitalization, and number of episodes.

**Participants**

A convenience sample of 60 patients diagnosed with major depressive disorder (MDD) from the Psychiatric Outpatient Clinic in Al-Zahra Hospital, Cairo, Egypt. The diagnosis was based on the DSM 5 criteria for MDD. A cross-matched control group comprised 60 subjects were selected from employers working in the hospital. All subjects provided written informed consent, approved applying personal data for research. The study was done consistent with good clinical practice and Declaration of Helsinki and the World Health Organization guidelines.

**Inclusion criteria**

The following criteria were included in the study:

1. Age range between 18 and 55.
2. Both sexes were included.

**Exclusion criteria**

The following criteria were excluded from the study:

1. Comorbid psychiatric disorders.
2. Medical illnesses such as endocrine, metabolic, neurological, or autoimmune disorders.
3. Infections, inflammatory reactions, or allergy within the past 2 weeks preceding blood sampling or antioxidant medication.

**Measures**

All subjects were subjected to the following:

1. Complete psychiatric sheet and examination and diagnosis were based on DSM 5 criteria for diagnosis of MDD.
2. Hamilton depression rating scale (HAMD) [20], its Arabic version [21]: A useful scale used to determine patients’ severity of depression.
3. Serum levels of MDA and SOD were estimated using Biodiagnostic kit, Egypt.
4. Serum Nrf2 levels was determined using Elabscience Biotechnology Co. Ltd. Enzyme-linked immunosorbent assay (ELISA) kit.

**Procedure**

Blood samples were taken from both groups under sterile conditions. Serum levels of MDA and SOD were estimated according to the methods described by Misra and Fridovich [22] and Ohkawa et al. [23], respectively.

Serum Nrf2 levels was determined using Elabscience Biotechnology Co. Ltd. ELISA kit. We followed the manufacturer’s instructions for definition and calculating results. Standards and samples were pipetted into wells with immobilized antibodies specific for human Nrf2, and then were incubated. After incubation and washing, biotinylated antihuman Nrf2 antibody was added. Having washed away any unbound substances, biotinylated antibody and horseradish peroxidase-conjugated streptavidin were pipetted into the wells, which were washed once again. tetramethylbenzidine substrate solution was added to the wells; color developed proportionally to the amount of Nrf2 bound. Color development was discontinued (Stop Solution) and its intensity was measured using the Thermo Labystems Multiskan Ascent 354 (Lab Recyclers) at 450 nm.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS version 21.0) was used for data entry and analysis. Descriptive statistics were computed in the form of frequency and percentage for categorical data and in the form of measures of central tendency (arithmetic mean) and measures of dispersion (standard deviation) for continuous variables. Chi-square test was used to test for the association and/or difference
between categorical variables. Student’s t-test was used for comparing two means and ANOVA for comparing more than 2 means. Multiple comparisons among sample means were checked by post hoc LSD. Pearson’s correlation coefficient (r) or spearmen’s rank order correlation coefficient was used for testing the association between two variables. Differences were considered as statistically significant when p = 0.05.

## Results

The socio-demographic data showed that cases were matching with the controls, no statistical difference between patient and control groups was observed regarding age, sex, education, and marital status (Table 1).

### Table 1: Socio-demographic characteristics of both patients and control groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient group n=60</th>
<th>Control group n=60</th>
<th>Sig test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean±SD)</td>
<td>41.03 ± 4.46</td>
<td>42.8 ± 4.26</td>
<td>t-test=2.2 df=118</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>18</td>
<td>22</td>
<td>χ²=0.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Females</td>
<td>42</td>
<td>38</td>
<td>df=1</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>34</td>
<td>30</td>
<td>χ²=3.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Secondary</td>
<td>18</td>
<td>16</td>
<td>df=4</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>8</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>4</td>
<td>10</td>
<td>χ²=6.23</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Married</td>
<td>36</td>
<td>28</td>
<td>df=6</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>14</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>6</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to HAMDS, the number of patients with mild depression was 28 (46.7%), moderate depression was 18 (30%), and severe depression was 14 (23.3%). The mean duration of illness in years was 6.13 and the mean number of episodes was 3.66 (Table 2).

### Table 2: Severity of depression according to Hamilton depression rating scale and disease characteristics

<table>
<thead>
<tr>
<th>Disease characteristics</th>
<th>Patient group n=60</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDRS</td>
<td>n (%)</td>
</tr>
<tr>
<td>Milder depression (%)</td>
<td>28 (46.7)</td>
</tr>
<tr>
<td>Moderate depression (%)</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Duration of illness (Mean ± SD)</td>
<td>6.13 ± 2.4</td>
</tr>
<tr>
<td>Number of episodes (Mean ± SD)</td>
<td>3.66 ± 1.43</td>
</tr>
</tbody>
</table>

Comparison between patient and control groups regarding MDA, SOD, and Nrf2 showed that the MDA level was higher in the patient group than the control one with statistical significance; meanwhile, SOD and Nrf2 were significantly lower in the patient group than the control one (20.46 ± 2.55 pg/ml, 42.2 ± 23.8 μM, 0.4 ± 0.1 pg/ml vs. 12.74 ± 0.26 pg/ml, and 131.02 ± 17.45 μM, 1.02 ± 0.04 pg/ml, respectively) (Table 3).

### Table 3: Comparison between patient and control groups regarding MDA, SOD, and Nrf2

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Control group n=60</th>
<th>Patient group n=60</th>
<th>Sig test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>12.74 ± 2.06</td>
<td>20.46 ± 2.55</td>
<td>t-test</td>
<td>0.001</td>
</tr>
<tr>
<td>SOD</td>
<td>131.02 ± 17.45</td>
<td>23.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nrf2</td>
<td>1.02 ± 0.04</td>
<td>0.4 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde, SOD: Superoxide dismutase, Nrf2: Nuclear factor erythroid 2-related factor 2.

Comparison between mild, moderate, severe depression, and control groups regarding MDA, SOD, and Nrf2 showed that patients with severe depression had significantly higher levels of MDA than those with mild depression; they also have significantly lower levels of SOD and Nrf2 than those with moderate depression and mild depression, respectively (Table 4).

### Table 4: Comparison between mild, moderate, severe depression, and control groups regarding MDA, SOD, and Nrf2

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Control group n=60</th>
<th>Mild depression</th>
<th>Moderate depression</th>
<th>Severe depression</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>12.74 ± 0.26</td>
<td>18.8 ± 2.90</td>
<td>21.79 ± 0.43</td>
<td>22.08 ± 1.73***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SOD</td>
<td>131.02 ± 17.45</td>
<td>56.03 ± 14.68</td>
<td>46.99 ± 18.54</td>
<td>8.4 ± 1.73***</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nrf2</td>
<td>1.02 ± 0.04</td>
<td>0.48 ± 0.099</td>
<td>0.35 ± 0.089</td>
<td>0.32 ± 0.05***</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Significantly different from control, Significantly different from mild, Significantly different from moderate, post hoc LSD at p<0.05. MDA: Malondialdehyde, SOD: Superoxide dismutase, Nrf2: Nuclear factor erythroid 2-related factor 2.

Relationship between MDA, SOD, Nrf2, and other variables revealed that there was strong positive correlation between MDA, SOD, Nrf2 levels, and duration of illness in years and number of episodes and no correlation was found between MDA, SOD, Nrf2, and age and gender (Table 5).

### Table 5: Relationship between MDA, SOD, Nrf2, and some variables (Age, gender, duration of illness, and number of episodes) in patient group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients (Age, gender, duration of illness, and number of episodes) in patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age and MDA</td>
<td>0.05</td>
</tr>
<tr>
<td>Gender and MDA</td>
<td>0.21</td>
</tr>
<tr>
<td>Duration of illness and MDA</td>
<td>0.76</td>
</tr>
<tr>
<td>Number of episodes and MDA</td>
<td>0.81</td>
</tr>
<tr>
<td>Age and SOD</td>
<td>0.23</td>
</tr>
<tr>
<td>Gender and SOD</td>
<td>0.06</td>
</tr>
<tr>
<td>Duration of illness and SOD</td>
<td>0.72</td>
</tr>
<tr>
<td>Number of episodes and SOD</td>
<td>0.73</td>
</tr>
<tr>
<td>Age and Nrf2</td>
<td>0.23</td>
</tr>
<tr>
<td>Gender and Nrf2</td>
<td>0.11</td>
</tr>
<tr>
<td>Duration of illness and Nrf2</td>
<td>0.78</td>
</tr>
<tr>
<td>Number of episodes and Nrf2</td>
<td>0.84</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde, SOD: Superoxide dismutase, Nrf2: Nuclear factor erythroid 2-related factor 2.

### Discussion

Recent researches showed that major depression is associated with activation in immune-inflammatory markers and there is an evidence that this activation is related to overproduction of ROS [24]. Some studies reported an increase in catabolism (oxidation) of monooamine neurotransmitters in MDD. It was also hypothesized that alterations in some lipids lead to changes in serotonin and noradrenaline which is thought to be related to depression [25]. Moreover, an established relationship between lipid peroxidation and ROS is found [26].

In our study, we examined the levels of MDA, SOD, and Nrf2 in the sera of 60 depressed patients and 60 controls and we found that MDA was significantly higher in patients than the control (20.46 ± 2.55 pg/ml...
vs. 12.74 ± 0.26 pg/ml) and these results are consistent with those of Billici et al. [27] who included 30 depressed patients and 30 controls and found that plasma MDA was significantly higher in depressed patients than controls (4.82 ± 1.3 µmol/L vs. 2.89 ± 1.1). Moreover, they found a positive correlation with its plasma level and both the number of episodes and duration of illness (r = 0.28, p < 0.05).

Likewise, Bajpai et al. [28] examined the MDA level in 60 depressed patients and 40 controls and found that its level was significantly higher in patients than controls (1.95 ± 1.04 mmol/L vs. 0.366 ± 0.175).

Similarly, Camkurt et al. [29] found that MDA level was significantly higher in patients with major depression than controls with medians at 4.04 nmol/mg and 1.64 nmol/mg, respectively. They also examined SOD which was significantly decreased in depressed patients than controls with means at 143 µ/mg and 298.12/mg, respectively, p < 0.001 and this matches with our study in which the SOD level was significantly less in patient group than the control one (42.2 ± 23.8 vs. 131.02 ± 17.45). Bajpai et al. [28] found that MDA was higher in patients than the controls (1.95 ± 1.04 mmol/L vs. 0.366 ± 0.175) and SOD was lower in patients (0.123 ± 0.068 µg/ml vs. 0.177 ± 0.042).

Likewise, several studies suggested elevated MDA levels in depression and a reduction following antidepressant therapy was documented by Mazereeuw et al. [30].

The present results suggest that oxidative stress, which is evidenced by elevated MDA marker levels, is disturbed in depressed patients.

Regarding Nrf2, in our study, we found that Nrf2 was significantly decreased in depressed patients than controls. A recent study showed the reduced (~21%) expression of Nrf2 in the dorsolateral prefrontal cortex from MDD patients [18].

These results suggest that decreased Keap1-Nrf2 signaling plays a key role in the pathophysiology of mood disorders such as MDD and bipolar disorder [31].

Only few studies have evaluated oxidative stress and the severity of depression.

In our study, patients with severe depression had significantly higher levels of MDA than those with mild depression. This was consistent with the study of Rangaswamy and Swath [32] who found that there was moderate positive correlation between MDA levels and clinical severity of depression as measured by 21-item Hamilton rating scale for depression score which was found to be statistically significant (r = 0.317, p = 0.025), and the study of Kotan et al. [33] who found that there was a positive correlation between the severity of depressive symptoms and SOD activity. This was in contrary to Bal et al. (2012) [34] who studied 42 patients (37 women, and 5 men) diagnosed with MDD and no correlation was found between HAMD scores and MDA at patient group. Furthermore, our study revealed significantly lower levels of SOD and Nrf2 in patients with moderate depression and mild depression, respectively, compared to control group. This was consistent with the study of Rawdin et al. [35] who found that Nrf2 did differ along the continuum of depressive symptom severity across groups. Furthermore, Sarandol et al. [36] found a positive correlation between the severity of depressive symptoms and SOD activity exists.

Researchers have proposed that oxidative stress is one of the potential pathogenic mediators for depression because it easily affects neuronal cell functions of the brain and hence the relation is settled between oxidative stress and depressive symptom scores.

In the current study, there was a significant relationship between the duration of illness and number of episodes and both high levels of MDA and low levels of SOD and Nrf2 with strong positive correlation. This was consistent with the previous studies such as Billici et al. [27], Stefanescu and Ciobica (2012) [37].

This can be attributed to explanations proven by recent research which stated that inflammation and mitochondrial oxidative process which occur in depression generate free radicals excessively with subsequent increase in MDA, these species react with macromolecules of the cell such as polyunsaturated fatty acids, DNA, proteins, and hence, damaging them [28], [38]. Therefore, definitely, the more the duration of illness, the more the number of episodes, the more free radicals and tissue destruction with higher levels of MDA.

Hence, to enhance personalized treatment for depressed patients, accurate prediction and detection of factors contributing to severity and recurrence of episodes is mandatory.

Conclusion

Our results indicate that oxidative stress can affect MDD, as free radicals are generated together with antioxidant deficiency; this can contribute to the occurrence of MDD and gives us a better understanding of the pathophysiology of depression and hence developing new therapeutic strategies.

Acknowledgment

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References

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PMid:28612257

PMid:17767685


PMid:14679177

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