Investigation the Relative Expression of Genes of ATPase Family in Major and Regular Depressed Patients

Sima Farzaneh¹*, Tavkol Moussazadeh², Mohammad Zaefizadeh³

ABSTRACT
To develop new pharmaceutical strategies in a comprehensive manner, it is necessary to understand the reasons for major depression. Present paper aims for comparison of expression of ATPase families in major depressed and normal people. Statistical population of this research includes all depressed people coming to professional clinic of Shahryar in 2014-15. This contains 47 depressed patients which were selected based on accessibility from those visiting clinic. 26 normal people were selected as comparison group as well. To study the relative expression of ATPase1-3, quantitative measurement method using Real-Time RT-PCR was used. Relative expression of ATPase2 showed a significant reduction compared to normal people (p<0.02). In patients, ATPase1 had higher relative expression compared to normal people. However, ATPase had no significant difference among patients and normal people. Results demonstrate that ATPase1 and ATPase2 are important variables in depression but ATPase3 plays no pivotal role in this regard.

Keywords: Major Depression, Gene Expression, ATPase

Major Depression Disease (MDD) is a frequent psychological disease which is predicted to be the second major factor of disability all around the world up to 2020 (Marie et.al, 1996, Ester et.al, 2010). WHO reported that currently, more than 450 millions of people the majority of which are living in developing countries suffer from psychological diseases or disabilities (Zamanlou et.al, 2013). This is accompanied with increase in deaths specially suicide (Harris et.al, 1998, Schneider et.al, 2001).

Depression is the most common neurotic dysfunction (Shamlou, 1998). In limited medical context, this means a behavioral disease or reaction dysfunction and in clinical context, it is a

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The objective of this research is to compare the level of ATPase genes expression in depressed and normal people. Depression is one of four main diseases of the world and the most common reason of disability resulted from diseases (Buchanan, 2012). Recent studies revealed that major depression has the highest frequency among psychological diseases (about 17%) (Saduk, 2010). One out of five people will experience major depression in a point in life (Kessler et.al, 2003). In recent years, in young population even in adolescents this disorder has increased considerably (Jean et.al, 2012). Episodic Major Depression is recognized with low temper and disability in enjoy or both for two weeks (Morgen et.al, 2009). In this case, person has a seriously depressed temper, psychological and movement laziness, anxiety, sadness feeling, embarrassment, sense of sin and suicide motivations (Saduk, 2007). Of the most devastating consequences of this disorder is reduction of tendency for work and activity (Hosseini et.al, 2012). Evidences show that various factors contribute to depression disease and a unique and clear factor cannot be considered as the sole factor of this problem.

Various variables such as age, social and personal factors, biological variables such as genetic diseases, hormonal disorders and drugs are among factors contributing to this disease. In studying the genetic structures contributing to depression disorder, majority of researches emphasize on the role of various genes in increasing the probability of depression (Zamanlou et.al, 2013). Biological pattern states that depression is a motivational disorder resulted from the lack of biogenic amines. Biogenic amines are neurochemical substrates which facilitate the
neural transfer. It is possible that depression resulted from reduction of Catecholamine including nor epinephrine, epinephrine, dopamine or one of Indole Amines such as serotonin and histamine (Rouznahan, 2007).

Therefore, present paper intends to answer the main question that whether there is any difference between ATPase gene expression of patient and normal people.

**ATPase family genes**

Na/K/ATPase protein is a membranous transport protein having the primary physiological role in survival of cellular transport of Na and K moving ions (Heurisberger, 2009) which is essential for control of stagnant membrane potential, cellular volume, cellular pH, K concentration and transport of other necessary solutions. Na/K/ATPase protein is a function of two subsections called α and β having several isoforms and distribution of patterns in various textures (Blanco and Mercer, 1998). Main function of α subsection is for hydrolyzing paired ATP by transporting K/Na. three forms of α isoforms are available as α₁, α₂ and α₃ each of which are codes for ATP1A1, ATP1A2 and ATP1A3, respectively. Central system expresses the isoforms of each part. (Heurisberger et.al, 2009). Previous studies illustrated that preventing activity of Na/K/ATPase reduces the recognition of norepinephrine, serotonin and dopamine (Stephens and Feveras, 2004) and therefore, this changes the neural cell communication (Valindo et.al, 2002). It is assumed that depression affects central neural network and then sympathetic and parasympathetic system and neuroendocrine system (axis of hypothalamus – adrenal pituitary) leading to abnormalities in transportation of serotonin – marking calcium and neurotropic agents (Lega et.al, 2008).

Studies have shown that there is a considerable reduction in ATP1A1 gene expression in majority of depression disorders compared to normal people which may reflect the change in level of this gene expression in central neural network (Lega et.al, 2008). Significant reduction in gene expression may have various effects on functionalities of the central neural network. Reduction in activity of Na/K/ATPase directly affects the signaling of neural transmitter of neural activity and all body movements through reduction of norepinephrine, dopamine, serotonin and increase of free acetyl choline (Watta and Stephens, 2004, Blassy et.al, 1988). By comparing isoforms of Na/K/ATPase subsections, it was discovered that brain addresses amino acid sequences and potential structures of protein. Investigation of criteria and tools reveals the contribution of isoforms of Na/K/ATPase subsections in various types of depression. Recent results led to development of the method of molecular recognition for major depression disease based on application of ATP1A1 as a molecular marker.
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MATERIALS AND METHODS

After determination of people of major depression group, they were approved through depression test carried out by a depression psychologist and for sampling, written consent was taken from them.

After approval of psychologist and getting consent, 3cc blood was taken from them and added to 15cc falcon containing 0.5cc EDTA anticoagulant (0.05M). Then, it was inverted so that it can be well mixed.

For RNA extraction, this must be performed 3 hours after blood taking. In this experiment, Roche kit made in Germany was used.

First step in this research was to separate WBCs. For this, according to kit instructions, 500μl blood containing EDTA (0.05M) was transferred to vial 1.5 and free RNase and DNase. Two times of blood volume, RCLB was added.

This vial was inverted for 10 minutes and then centrifuged by 2500rpm for 5 minutes. After centrifuge, above solution was discarded and only deposits were kept. Again, 1000μl RLCB was added to vial and inverted for 5 minutes. After mixing, it was centrifuged for 3 minutes by 2500rpm. Then, above solution together with RBCs surrounding white deposition was removed from the vial and 200μl PBS 1x was added. After removal of RBCs, kit can be used for extracting RNA.

Next step of experiment is to extract RNA from RBCs. First, 400μl cell lysis binding was added to vial contacting 200μl PBS 1x and it was suspended slowly and vortexed for 15s to obtain a clear and homogeneous solution. Then, this solution was added to vials having filters and centrifuged for 15s in 10000rpm. After centrifuge, below solution was removed. In this stage, RNA and DNA stick to the filter. Then, 90μl incubation buffer and 10μl DNase I were added to vial.

After adding DNase, vials were put under hood for 15 minutes to affect DNAs. Then, 500μl solution of Wash Buffer I was added to filters and centrifuged for15s in 10000 rpm. This was done for washing out salts, proteins and so on. Then, below solution was removed. Next stage was adding 500μl Wash Buffer II and centrifuging for 15s in 10000 rpm. Then, below solution was removed. For the next time, Wash Buffer II as much as 200μl was added to vial and then, it was added to vial and centrifuged for 2 minutes in 13000 rpm. Then filtered vial, was sterile and free RNase and DNase were transferred. After transferring, 50-100μl elution buffer was added to vial filter and centrifuged for 1 minute in 10000 rpm. In this stage, below solution entering vial contains RNA. Finally, vial was stored in -70ºC.
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Evaluation of purity and quality of extracted RNA
After extracting mRNA, its quality and quantity was assessed using spectrophotometry and electrophoresis of Agarose gel.

Data analysis method
To investigate hypotheses, in descriptive statistics, central indices and dispersion corresponding to questionnaire scores were determined. In this section, t-test method was used for investigation of hypotheses and $2^{-\Delta\Delta CT}$ formula was used for determination of changes in genes expression. Level of significance for testing hypotheses was set less than 0.05. To analyze data, SPSS 16 was used.

RESULTS AND DISCUSSION
According to table 1, it was revealed that average age of normal people in this work is 39.12 with 1.89 standard deviation. Patients were slightly younger and their average age was 37.71 with standard deviation as much as 4.99. Average of ATPase1 of relative expression in normal people was 0.44 with 0.841 standard deviation. On the other hand, average of ATPase1 of relative expression in patient people was 2.06 with 1.21 standard deviation. Average of ATPase2 in normal people was 1.79 with 2.65 standard deviation; while the average of ATPase2 in patient people was 2.27 with 1.42 standard deviation. Finally, Average of ATPase3 in normal people was 2.62 with 1.24 standard deviation, while average of ATPase3 of relative expression in patient people was 2.098 with 1.024 standard deviation which shows no significant change between groups.

Table 1: average and standard deviation of age for normal and patient people

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depression</th>
<th>Age</th>
<th>ATPas3</th>
<th>ATPase1</th>
<th>ATPase2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>14.46</td>
<td>39.12</td>
<td>2.62</td>
<td>1.44</td>
<td>1.79</td>
</tr>
<tr>
<td>Abundance</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>3.45</td>
<td>1.89</td>
<td>1.24</td>
<td>0.842</td>
<td>2.65</td>
</tr>
<tr>
<td>Patient</td>
<td>46.29</td>
<td>31.71</td>
<td>2.09</td>
<td>2.06</td>
<td>1.27</td>
</tr>
<tr>
<td>Abundance</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>5.75</td>
<td>4.99</td>
<td>1.02</td>
<td>1.21</td>
<td>1.42</td>
</tr>
<tr>
<td>Sum</td>
<td>38.52</td>
<td>38.21</td>
<td>2.28</td>
<td>1.84</td>
<td>1.46</td>
</tr>
<tr>
<td>Abundance</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>11.66</td>
<td>4.20</td>
<td>1.12</td>
<td>1.12</td>
<td>1.95</td>
</tr>
</tbody>
</table>
Analytical findings

**Table 2: average and results of t-test for relative scores of studied genes for normal and patient people**

<table>
<thead>
<tr>
<th></th>
<th>Abundance</th>
<th>Amplitude</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
<th>Standard deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>73</td>
<td>35</td>
<td>20</td>
<td>55</td>
<td>38.52</td>
<td>1.36</td>
<td>11.66</td>
</tr>
<tr>
<td>Age</td>
<td>73</td>
<td>23</td>
<td>22</td>
<td>45</td>
<td>38.21</td>
<td>0.49</td>
<td>4.20</td>
</tr>
<tr>
<td>ATPase3</td>
<td>73</td>
<td>3.54</td>
<td>0.87</td>
<td>4.32</td>
<td>2.28</td>
<td>0.13</td>
<td>1.12</td>
</tr>
<tr>
<td>ATPase1</td>
<td>73</td>
<td>3.71</td>
<td>1</td>
<td>4.71</td>
<td>1.84</td>
<td>0.13</td>
<td>1.12</td>
</tr>
<tr>
<td>ATPase2</td>
<td>73</td>
<td>8.87</td>
<td>0.06</td>
<td>8.93</td>
<td>1.46</td>
<td>0.22</td>
<td>1.95</td>
</tr>
<tr>
<td>Abundance</td>
<td>73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In table 2, average scores of patients according to questionnaire is 46.29±5.75 for depression, 1.06±1.12 for ATPase1, 0.27±1.42 for ATPase2 and 2.09±1.02 for ATPase3. Moreover, it was 24.46±3.45 for depression, 2.44±0.84 for ATPase1, 1.79±2.65 for ATPase2 and 2.09±1.02 for ATPase3 in normal people.

**Table 3: average and standard deviation for normal and patient people**

<table>
<thead>
<tr>
<th>P_{value}</th>
<th>DF</th>
<th>t</th>
<th>Standard Deviation</th>
<th>Average</th>
<th>Group</th>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>71</td>
<td>-17.645</td>
<td>5.75</td>
<td>46.29</td>
<td>Patient</td>
<td>Depression</td>
</tr>
<tr>
<td>0.000</td>
<td>71</td>
<td>3.45</td>
<td>14.46</td>
<td></td>
<td>Normal</td>
<td>ATPase1</td>
</tr>
<tr>
<td>0.024</td>
<td>71</td>
<td>-2.311</td>
<td>0.08</td>
<td>2.44</td>
<td>Patient</td>
<td>ATPase2</td>
</tr>
<tr>
<td>0.013</td>
<td>71</td>
<td>0.84</td>
<td>1.06</td>
<td></td>
<td>Normal</td>
<td>ATPase3</td>
</tr>
<tr>
<td>0.028</td>
<td>71</td>
<td>1.073</td>
<td>1.42</td>
<td>0.27</td>
<td>Patient</td>
<td></td>
</tr>
<tr>
<td>0.036</td>
<td>71</td>
<td>2.65</td>
<td>1.79</td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>0.077</td>
<td>71</td>
<td>1.933</td>
<td>1.02</td>
<td>2.09</td>
<td>Patient</td>
<td></td>
</tr>
<tr>
<td>0.074</td>
<td></td>
<td>1.24</td>
<td>2.62</td>
<td></td>
<td>Normal</td>
<td></td>
</tr>
</tbody>
</table>

Depression average in patients was significantly higher than normal people (P<0.000). Level of gene expression for ATPase2 in patients showed a significant reduction compared to normal people (P<0.287). ATPase1 had a significantly higher gene expression in patients (P<0.024). However, ATPase3, showed no significant difference among patients and normal people. it seems that expression of this gene has no contribution to depression.

Relative expression of ATPase was measured using Real-Time PCR with respect to GADPH gene expression and using Pfeufa formula with efficiency as much as 1.97. CT results are illustrated in Fig. 1.
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**Fig. 1: logarithmic phase of ATPase3 gene expression using RT-PCR**

In Fig. 2, approaching premature logarithmic phase represents the higher relative expression since Sybr Green attaches to two strands of DNA and as initial expression of mRNA increases, cDNA level will increase as well and attaches Sybr Green faster and will be visible. Therefore, MT in 83°C will be seen sharply and this illustrates the efficiency of PCR in recognizing expression values.

**Fig. 2: TM illustration for three studied genes**

Comparison of relative expression of ATPase2 is shown in Fig. 2. Results demonstrated that relative expression in MDD patients decreased significantly (P<0.02) which reveals the reduction of ATPase receptor expression as a result of disease.
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**Fig. 4: CT (Logarithmic Fase) For relative Expression of ATPase1-2 genes**

Relative expression of ATPase with respect to control group was investigated and it was revealed that ATPase1 reduced from 1 to 0.3 and this reduction is significant. However, ATPase3, showed no significant reduction and ATPase2 reduced from 1 to -1.3 which is a significant reduction and demonstrates that ATPase2 has a pivotal role in depression.

Relative comparison of ATPase1 is shown in Fig. 4. Results revealed that relative level of expression in MDD patients has increased significantly. Relative expression of ATPase1 was measured using Real-Time PCR with respect to GADPH gene expression and using Pfeufa formula with efficiency as much as 1.97.

In Fig. 4, approaching premature logarithmic phase represents the higher relative expression since Sybr Green attaches to two strands of DNA and as initial expression of mRNA increases, cDNA level will increase as well and attaches Sybr Green faster and will be visible. Therefore, MT in 53°C will be seen sharply and this illustrates the efficiency of PCR in recognizing expression values.

Comparison of relative expression of ATPase3 is shown in Fig. 1. Results revealed that relative expression of this gene shows no significant difference between patients and normal people (P<0.07) which represent the indifference of receptor of ATPase3 as a result of disease. Results reveal the reduction of expression of ATPase2 in patients compared to normal people. In addition, in ATPase1, relative expression increased but there was no significant difference between two groups which seems that this gene has no contribution to depression.

**CONCLUSION**

According to results, it was confirmed that relative expression of ATP1 and ATP2 decreased significantly in MDD patients. According to research hypothesis, it can be claimed that there is a significant difference in gene expression of ATP1 and ATP2 between patients and normal people. Results of this work are consistent with findings of Lili et.al who studied 22-26 years old Chinese people (19 men and 11 women) except that in their studies, relative expression of
ATPase2 was 0.8 while in this work it is as much as 0.23. This demonstrates the contribution of ATPase2 in depression. According to Fig. 3, relative expression of ATPase1 decreased significantly which means that this gene plays role in depression as well.

Major depression affects central neural network and then autonomous nervous system, immunological system and endocrinology glands. Na/K/ATPase as a great intermediate for ion movements in cellular membrane transportation, plays an important role in transferring neural signal. Three types of Na/K/ATPase are discovered in brain but they vary among cells and expression level. It is confirmed that reduced gene expression for ATP1A2 and ATP1A3 are related to depressant dysfunction. However, there is no reported relationship between ATP1A1 and depression. This paper studied the potential relationship between expression level of ATP1A1 and major depression. Expression level for this gene was limited by surrounding flow in both depressed and normal people and controls of normal person was limited by translated quality polymerase chain reactions. Statistical analysis shows a significant decrease in expression of ATP1A1 in most of the depressed patients which is compared with health control (P<0.01). Difference of nucleotide genes order and that of protein structures were expressed as well. These investigations proved for the first time that expression of ATP1A1 gene is strongly correlated with depression. It is suggested that ATP1A1 could be a molecular sign for recognition of the disease (Lili et.al, 2012).

The main role of ATPase is forming Na⁺/K ion channel for transferring ATP through mitochondria of cytosol environment and ultimately supplying energy of metabolite processes (Rajakusked et.al, 2007). According to results, it seems that the level of energy produced in depressed people is lower since ATPase1 and ATPase2 have lower relative expression. Therefore, it can be said that even in production of ATP in mitochondria, its transfer to cytosol channels are less produced. Hence, ATP required by cell will decrease.

This finding is in agreement with Zamanlou et.al (2013) about relative expression of COX. In addition, lower energy level is reported in MS patients as well (Safavi et.al, 2013). Presence of a significant relationship between depression and relative expression of ATPase1 and ATPase2 justified the same outcome.

In present work, in relative expression of ATPase1 in depressed people, 230% reduction compared to normal people was observed. This is in agreement with Lili et.al (2013). By investigating the major depression, they demonstrated that there is more than 80% reduction in expression of ATP1A1 in depressed patients compared to control group; while reference controlling gene, β-actin showed no significant changes in each group. Unique results of variance of two groups of t-test, revealed the statistical differences. Another study was performed in bioinformatics which shows the comparison of structure of genes, comparison of
nucleotide sequence of each of isoforms from recognition of sequence of ATP1A1 and ATP1A3 genes. However, no significant similarity was observed between ATP1A1 and ATP1A2. Nevertheless, comparison of amino acids sequences, revealed 87% of recognitions between ATP1A1 and ATP1A2 or ATP1A1 and ATP1A3. All three isoforms of transporting membrane and coiled-coiled regions showed the same functionality (Oh et.al, 2012).

Acknowledgments
The author appreciates all those who participated in the study and helped to facilitate the research process.

Conflict of Interests
The author declared no conflict of interests.

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How to cite this article: S Farzaneh, T Moussazadeh, M Zaefizadeh (2016), Investigation the Relative Expression of Genes of ATPase Family in Major and Regular Depressed Patients, International Journal of Indian Psychology, Volume 3, Issue 4, No. 56, ISSN 2348-5396 (e), ISSN: 2349-3429 (p), DIP: 18.01.014/20160304, ISBN: 978-1-365-23992-2