Plasma Brain Derived Neurotrophic Factor Level and Its Gene Polymorphism in Toxic Suicidal Behavior

Mohammad A. El-Kattan¹, Ahmed M.N.Z. Helaly¹, Zakaria F. Lotfy²,
Adel M. El-Mansoury¹, Samia A. Hassan¹

ABSTRACT

Suicide represents a major health problem, it is suggested that multiple factors influence the rate of suicide. Brain derived neurotrophic factor (BDNF) is considered a reliable biological marker of suicide. Its level decreases in all suicidal victims whether or not they are suffering from psychiatric troubles. The objective of this work was to compare BDNF levels and single nucleotide polymorphism in suicidal and non-suicidal groups to explore whether suicide is related to BDNF related mechanisms. Plasma BDNF levels were screened in conjunction with tracking of the 196 G/A (val66met, rs6265) polymorphism in 89 suicidal patients that visited the toxicology unit and 89 control. The pattern of gene polymorphism did not show significant differences between suicidal patients and control. However, the plasma BDNF levels were higher in suicidal cases. It can be concluded that the combined assessment of BDNF showed no difference in genotyping between suicide and control groups. However, higher levels of plasma BDNF were detected in the samples taken from suicide attempters.

Introduction

Suicidal behavior (SB) is considered a major health challenge. According to WHO studies, up to 2% of the deaths in the world are caused by unfortunate successful suicidal attempts that represent the 10th leading cause of death in the world. It is expected that 1.5 million will die as a result of suicide in the next 10 years all over the world (WHO, 2002). Multifactorial causes are likely to influence the tendency to suicide for example biological, genetic and psychosocial factors (Hawton and Van Heeringen, 2009).

Neurotrophins are responsible for regulation of structural, synaptic, and morphological plasticity of the central nervous system. Furthermore, they support the function and response of the synaptic connections and neurotransmission. Brain derived neurotrophic factor is one of the most important extensively studied brain growth factors. It is the ligand binding to a tropomyosin-related kinase B (TrkB) receptor and is considered a reliable biological marker of suicide. Its level decreases in all suicidal victims whether or not they are suffering from predisposing psychiatric troubles (Paska et al., 2013).

A low level of BDNF is associated with serotonergic abnormalities in young suicidal patients corresponding to possible defective brain development. It is worthy to say that serotonin dysfunction is a cornerstone factor in the pathogenesis of anxiety and depression (Dwivedi, 2012).

It is hypothesized that poor blood BDNF level is associated with poor neurogenesis in...
the hippocampus that explains the tendency to commit suicide. Many studies are supporting that stress as well as decreased BDNF level affects neuronal plasticity as well as neurogenesis (Van Heeringen et al., 2011).

The most extensive research had been done on a single-nucleotide polymorphism (SNP) (rs6265) in coding region of exon V of the BDNF gene, producing an amino acid substitution (valine to methionine) at codon 66 (Val66Met). This amino acid substitution corresponds to abnormal intracellular recruitment of BDNF. As a result, the production of the functional neurotrophin is reduced in the central nervous system (Czira et al., 2012).

The average serum levels of BDNF are above 100-fold higher than plasma levels. This result can be explained by degranulation of platelets in the process of clotting. Clinical studies reported that circulating plasma BDNF is considered a good biomarker for depression and suicide (Brunoni et al., 2008; Piccinni et al., 2008). It is important to add that BDNF originates from both neurons and astrocytes, passes the blood brain barrier using an active transport mechanism and plasma BDNF levels strongly correlate with cortical gene expression (Lang et al., 2007).

The aim of this work was to study the BDNF gene polymorphism in relation suicide and to check plasma levels of BDNF in the suicidal group. The combined parameters are expected to explain the role of BDNF in suicide.

Subjects and Methods

Subjects:

This is a case control study that was conducted from April 2015 to March 2016. The patients were recruited from the Toxicology Unit, Mansoura Emergency Hospital, Dakahleia Governorate, Egypt.

Subjects were divided into two groups: 89 suicidal cases and 89 controls of both sexes. The age range of the suicidal patients was from 15-60 years. The suicidal cases with or without psychiatric history were included. Smokers, drug abusers and patients taking antidepressant drugs were excluded. The control samples were taken from the same locality with exposure to the same variables like time at which the blood samples were taken, storage, psychosocial standard, age, sex, smoking habits, nutritional status or alcohol intake. They were living in the same geographic area and had the same ethnicity as patients. They had no clinical evidence or family history of suicidal attacks. The blood samples were collected, plasma were separated and kept frozen at – 80 ºC until the time of analysis.

The study was approved by the Research Ethics Committee (REC) for experimental and clinical studies at the Faculty of Medicine, Mansoura University, Egypt. Informed written consents were obtained from all subjects before starting the study. The study was performed according to the Declaration of Helsinki Principles.

Methods:

All studied subjects were subjected to the following:

1) Detailed history taking including a standardized questionnaire that was applied to confirm the actual suicidal behavior.

2) Blood sampling for estimation of plasma BDNF levels:

Samples collection:

Venous blood samples (2.5 ml) from patients and controls were obtained, preserved in ethylene diamine tetra acetic acid (EDTA) tubes and centrifuged at 4000 r.p.m for 15 min. The samples were taken 30 minutes after the patients of self-deliberate poisoning were
admitted to the Toxicology unit. The patient generally reached the hospital within the 1st to 6th hours after exposure to the acute poisoning.

- **Samples storage:**  
  Plasma components were stored at – 80°C until the time of assay.

- **Samples analysis:**  
  Brain derived neurotrophic factor levels were evaluated in both patients and controls by an ELISA method (Human BDNF Eliza kit, Sun Red Biotechnology, Shanghai). The BDNF protein was expressed as equivalent of the human recombinant protein; the normal range of plasma BDNF was 0.1-10 ng/ml.

3) **Blood sampling for genotyping:**  
Two and half (2.5) ml venous blood samples on EDTA containing tubes were collected from each subjects and left frozen at – 80°C until applying the genotype test for presence or absence of SNP: 196 G/A (val66met, rs6265).

A commercial DNA extract kit, Gene Jet Whole Blood Genomic DNA purification Mini kit (Thermo Scientific, EU Lithuania) was applied to extract DNA from blood leukocytes. Typing of the BDNF 196 G/A (rs6265) gene polymorphism was done according to Choo et al.; (2014). The primer sequences (Biosearch technologies, USA) used were as follows: the forward primer 5’- GAG GCT TGA CAT TGG CT-3’ and the reverse primer 5’- CGT GTA CAA GTC TGC GTC CT -3’. The reaction mixture volume was 25 uL and included 5uL of 100ng/ul DNA, 15.0 uL of DeamTaq Green Master mix (Fermentas, Germany, Lot No.39428), 0.5 μL of each primer and 4 uL of distilled water. PCR reaction conditions were done on a thermo-cycler PTC-100 (BioRad) to amplify the DNA for 35 cycles. After 10 minutes heating at 95° C, the cycle was divided into 30 seconds at 94°C, 30 seconds at 62°C, and 30 seconds at 72°C, and finally 5 minutes heating at 72°C. A total of 10 uL of the PCR products were resolved on 2% agarose gels to check the PCR products at the 113-bp fragment. RFLP analysis was done using Fast-Digest restriction enzyme NIaIII (Thermo Scientific, EU Lithuania) to cut the amplified DNA at the 196A site, and the product was evaluated by running electrophoresis in 3% agarose gels and stained with ethidium bromide. Homozygous genotypes were discriminated by the presence of 113bp bands (G/G) or bands of 75, 38 bp (A/A). The heterozygous genotype showed three bands: 113, 75, and 38 bp (A/G).

**Statistical analysis**

Statistical analysis of the data was performed using Excel (Microsoft Office 2013) and SPSS version 20 (SPSS, Inc., Chicago, IL, USA). Qualitative data were presented as number and percentage; X² and Fisher’s exact tests were used to compare groups. Quantitative data were presented as mean and S.D. or median and range. For comparison between the two groups, non-parametric Mann–Whitney and U-tests were used. Deviations from Hardy–Weinberg equilibrium expectations were determined using X² test. Odds ratio (OR) and 95% confidence interval (CI) were calculated. The correlations between different parameters were analyzed using Spearman’s rank correlation coefficient. Changes in BDNF protein concentration and antibody titer over time were examined by Wilcoxon’s paired signed-rank test. P value was considered significant if < 0.05.

**Results**

The pattern of gene polymorphism did not show significant differences between suicidal patients and control. However, plasma BDNF levels were higher in suicidal cases (Table 1). The median of BDNF plasma levels
was significantly higher in the suicide group with wider distribution in the control group (Figure 1). The median blood BDNF levels for AG and GG polymorphisms was 2.63 and 2.97 ng respectively in the suicide group while for the non-suicide group they showed a median of 2.32 and 1.99 ng respectively (Table 2). The BDNF levels range from 0.02 to 11.64 ng in suicidal AG genotype, and range from 1.89 to 13.51 ng in suicidal GG genotype. The range of BDNF plasma levels in non-suicidal AG group was 1.29-18.6 ng. The range of BDNF in non-suicidal GG genotype was 0.88 to 15.5 ng (Figure 2).

The pattern of gene polymorphism in both suicidal cases complaining of psychiatric diseases and those who committed suicide attempts with no history of psychiatric diseases was evaluated in table (3). The range of BDNF level in suicidal cases complaining of psychiatric troubles was 0.02 to 3.44 ng and the range in suicidal cases not complaining of diseases was 1.76 to 13.51 ng (Figure 3).

### Table (1): Comparison of single nucleotide polymorphism between suicidal and non-suicidal groups in relation to brain derived neurotrophic factor (BDNF) plasma level (n=178).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Suicidal group (n=89)</th>
<th>Non-suicidal group (n=89)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF level by ng/ml described as median (Min-Max)</td>
<td>2.72 (0.02-3.51)</td>
<td>2.11 (0.88-18.6)</td>
<td>Z = 5.28, p &lt; 0.001*</td>
</tr>
<tr>
<td>Single nucleotide polymorphism</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>AG **</td>
<td>40(40)</td>
<td>33(33)</td>
<td>χ² = 1.057, p = 0.304</td>
</tr>
<tr>
<td>GG</td>
<td>60(60)</td>
<td>67(67)</td>
<td></td>
</tr>
</tbody>
</table>

n: number, *Z: Mann Whitney test, χ²: Chi square test, **The heterozygous genotype (A/G), homozygous genotype (G/G).

**Fig.(1):** The distribution of median plasma brain derived neurotrophic factor (BDNF) levels in both suicidal and non-suicidal groups.
Table (2): Comparison of single nucleotide polymorphism and median brain derived neurotrophic factor (BDNF) plasma level between suicidal and non-suicidal groups.

<table>
<thead>
<tr>
<th>Gene Polymorphism</th>
<th>Suicidal group n = 89</th>
<th>Non-suicidal group n = 89</th>
<th>Mann Whitney test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG</td>
<td>2.63 (0.02-11.64)</td>
<td>2.32(1.29 -18.6)</td>
<td>Z=2.22</td>
<td>0.026</td>
</tr>
<tr>
<td>GG</td>
<td>2.97(1.89- 13.51)</td>
<td>1.99(0.88 -15.5)</td>
<td>Z=4.99</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

n: number

Fig. (2): Distribution of plasma levels of brain derived neurotrophic factor (BDNF) in suicidal versus non-suicidal groups in both AG and GG phenotypes.

Table (3): Comparison between suicidal group complaining of psychiatric disease versus non psychiatric suicidal group regarding blood brain derived neurotrophic factor BDNF levels and gene polymorphism.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Psychiatric diseases (n= 89)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (n =18)</td>
<td>Absent (n = 71)</td>
</tr>
<tr>
<td>BDNF</td>
<td>2.59 (0.02 - 3.44)</td>
<td>2.73 (1.76 - 13.51)</td>
</tr>
<tr>
<td>Gene polymorphism</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>AG</td>
<td>7 (40)</td>
<td>29 (40)</td>
</tr>
<tr>
<td>GG</td>
<td>11 (60)</td>
<td>42 (60)</td>
</tr>
</tbody>
</table>

n: number
Discussion

The present study examined the comparison between gene expression of BDNF and BDNF gene polymorphism. Both results challenged genetic SNP and protein expression in relation to suicide. The BDNF blood level was higher in suicidal group in contrast to solid uniform genetic background of BDNF gene polymorphism. No statistically significant distribution of gene polymorphism was detected in relation to suicide.

On the other hand, screening of BDNF blood levels showed dynamic response in the suicidal group where the median BDNF blood level was higher in individuals attempting suicide than in control group, with wider distribution. At the same time, suicidal patients with psychiatric manifestation revealed lower blood levels of BDNF than those attempting suicide without history of psychiatric diseases. Still, both suicidal groups expressed higher plasma BDNF than the control.

Studies showed that BDNF is a strong marker for stress and suicide (Lang et al., 2007; Brunoni et al., 2008; Piccinni et al., 2008). Post mortem analysis of mRNA of BDNF extracted from hippocampus showed a significant reduction in hippocampal BDNF and TrkB mRNA expression among individuals who died by suicide compared to normal controls (Smith et al., 1995). The hypothesis tracking brain or blood levels of BDNF suggests that neuronal plasticity plays a role in the pathogenesis of suicide. Such proposed pathology is associated with modifications of BDNF expression (Dunman et al., 2000; Garcia, 2002; Fossati et al., 2004).

Brain derived neurotrophic factor is an important growth factor regulating the growth of serotonin secreting neurons and mediates neuronal plasticity supporting the nervous system and combating stress either acute or chronic. Furthermore, there were published data indicating that BDNF expression decreases in patients having psychiatric disorders (Thompson et al., 2009). Combined suicide with history of...
psychiatric disorders supports the notion that BDNF is a reliable growth factor reflecting suicide and stress and it is considered as a biological marker for suicide whatever the cause is (Terraciano et al., 2011; Dwivedi et al., 2012; Ambraus et al., 2016).

As regarding genetic analysis of suicide, a wide genome analysis study revealed no strong genetic association with suicide, however, mRNA biomarkers showed that BDNF expression was affected making it shadow markers for suicide in affective disorders (Pulay and Rethelyi, 2016). Studies showed that committing suicide increases dramatically in psychiatric patients suffering from mental disorders including schizophrenia, bipolar or major depressive diseases, yet, the pathogenesis behind suicide in apparently normal subjects remains a mystery. It is suggested that multiple genetic interactions may be responsible for a neuro-developmental anomaly that is responsible for suicidal attempts later on (Sokolowiski et al., 2015).

In this study, BDNF values showed higher levels in the serum of apparently healthy suicidal group in contrast to suicidal patients with psychiatric disorders whereas the blood levels of BDNF were higher than the control. These biphasic comparative results could be explained by the theory that young apparently healthy suicidal patients are under stress that promotes defensive mechanisms to overcome the pressure, and by then the secured blood levels of BDNF reflect a compensatory mechanism to stressors causing these patients to commit suicide. Finally, the defensive mechanisms are expected to be exhausted in the psychiatric suicidal group where the levels of BDNF decline. This theory is supported by recent research done on Alzheimer’s patients who expressed high BDNF protein more than the control to overcome the process of degeneration (Faria et al., 2014).

A study showed that BDNF expression in an early epileptic animal model was higher in neo-cortex than the control. Later on, a chronic epileptic model revealed that BDNF expression was lower in the surrounding areas indicating plastic changes in the neurons (Liang et al., 1998). Studies suggested that high BDNF expression contributed to increased neuronal activity and associated with the development of epileptic focus despite its well-known cytoprotective function (Lindvall et al., 1994). It is difficult to conclude that suicide pathology expresses epileptic pattern despite that the epilepsy model for suicide attempt may be a new mechanism of suicide.

It is important that the locality in Mansoura, Egypt, may have a unique pattern of life style that enforces BDNF levels to increase in stressed young candidates. The rate of unemployment is very high. Such social burden induces stress on the central nervous system. A new study applied on mice showed that socially defeated wild animals expressed high levels of BDNF as a protective mechanism (Bartlang et al., 2016). From this notion it is suggested that BDNF can be a marker of prodromes of psychiatric disorders, especially if associated with suicidal events. New studies showed that high BDNF transcription is not always a sign of security. Patients with sleep disorder showed higher serum BDNF than the control (Wei et al., 2016).

Genetic linkage studies showed that the BDNF (Valine66/Met) variant is characterized by variable expression of BDNF and changes of the volume of the hippocampus (Egan et al., 2003; Chen et al., 2004; Toro et al., 2009). Both GG and AG genotypes express the same pattern of higher BDNF in apparent healthy suicide attempters. This finding postulated an epigenetic mechanism behind suicide; however, the study tracked only one SNP.

The absence of AA SNP in Egyptian samples, either suicidal or control, created a
different signature from other racial samples. It is suggested that GG/ GA may be associated with a different biochemical response and different BDNF blood levels in reaction to stress. New studies suggest other genes interacting with BDNF in the pathogenesis of suicide including cholecystokinin (CCK) and cholecystokinin beta-receptor (CCKBR) genes (Sears et al., 2013).

The suicidal cases with history of psychiatric disorders showed lower levels of BDNF than the apparently healthy group. This decline may correlate with the development of permanent neuronal plasticity. However, both groups were still higher than the control. These results reflect modulation of neuronal plasticity in suicide victims. Wider scale study and long-term assessment of these patients are needed in both GG and GA phenotypes to reevaluate the relation between BDNF and depression with or without suicide.

It is suggested that young suicidal patients still have the reserve to overcome depression with higher blood BDNF, and the hypothesis is that repeating blood evaluation of BDNF levels would show decrease with time, especially without antidepressant treatment.

Recent studies demonstrated that the polymorphism Val66Met is associated with suicidal attempts in depressed patients using drastic methods. Another study indicated that BDNF polymorphism increased the rate of suicide in men taking antidepressants through genetic modulation of the noradrenergic system leading to suicide. Despite the suggested cyto-protective effects of antidepressants and possible elevation of BDNF, the rates of suicide increased with antidepressant medication (Akiskal and Mallya, 1987; Reeves and Brister, 2008; Schenkel et al., 2010).

Conclusion

The present study showed unexpectedly higher blood levels in the samples taken from the suicidal group. This result can be explained by compensatory mechanisms to overcome stress related to life and economic styles in the Egyptian samples. The BDNF polymorphism, Val66Met, wasn’t correlated with a higher rate of suicide. All cases showed wild-type favorable genetic background. It is assumed that multiple models like genetic, epigenetic and epilepsy may be potential models for the pathogenesis of suicide. Larger samples and longer periods are needed to evaluate the relation between BDNF and suicide.

Acknowledgment

We would like to thank all members of the Immunology Unit, Clinical Pathology Department, Mansoura Faculty of Medicine, for their assistance in the lab work of this research.

References


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مستوى عامل التغذية العصبي في البلازما وتعدد أشكاله الجينية في السلوك التسميمي الانتحاري

محمد عبد السميع السيد القطن، أحمد محمد نبيل زكي هلال، زكريا لطفي، عادل محمود المنصوري، سامية أحمد حسن

من أقسام الطب الشرعي والسموم الأكلينيكية - بالانولوجيا الأكلينيكية - كلية الطب - جامعة المنصورة

يعتبر السلوك الانتحاري مشكلة صحية عامة ورئيسية، وهناك عدة عوامل تؤثر في الاستعداد للانتحار منعا للعوامل البيولوجية والوراثية والنفسية والاجتماعية. ويُعتبر عامل التغذية العصبي (BDNF) علامة بيولوجية موثقة للانتحار، حيث ينخفض مستوياته في جميع الضحايا المنتحرين سواء كانوا يعانون من مشاكل نفسية أم لا. إن الهدف من هذا البحث هو مقارنة مستويات عامل التغذية العصبي وتعدد أشكال النبوكلوتيدات المفردة في مجموعات المنتحرين و غير المنتحرين، واستكشاف ما إذا كان الانتحار يرتبط بأليات ذات الصلة بعامل التغذية العصبي. تم فحص مستوى عامل التغذية العصبي في البلازما بالإضافة إلى تتبع تعدد الأشكال الجينية (G/A196) G/A166met, (rs6265) في 89 من المرضى المنتحرين الذين ترددوا على وحدة السموم في مستشفى الطوارئ جامعة المنصورة و89 من المجموعة الضابطة. وقد أظهرت النتائج أن نمط تعدد الأشكال الجينية لم يظهر اختلافات كبيرة بين المرضى المنتحرين والمجموعة الضابطة. ومع ذلك، كانت مستويات عامل التغذية العصبي في البلازما أعلى في حالات الانتحار.