ÖZET
Ağrıli Sendromlardaki serum beyin-üçlüsü nörotrofi faktör düzeyleri: Major depresyon ile körülüğü bir çalışma

Amaç: Bu çalışmada, ağrıları sendromları ortaya çıkmak için serum froninının ağırlığının mide ve fibromiyalji tanısı hastalarının serum beyin-üçlüsü nörotrofi faktör (BDNF) düzeyleri depresif hastaların ve sağlıklı kontrollerin düzeyleri ile karşılaştırılacaktır.

Yöntem: Öncedden herhangi bir psikiyatrik tanısı olmayan ve antiparkinson tedavi kullanmamış olan 27 migen tanısı olan ve 19 fibromiyalji tanısı hastaların serum BDNF düzeyleri ölçülmüştür. Depresyon grubunda ise en az sekiz haftadır antidepresan tedavi kullanmamış maje depresyon etkenlerini içeren 24 hasta dahil edilmişdir. Bu grupta depresyona eşlik eden başka bir birincı tanısı olan hasta yoktut. Her hangi bir psikiyatrik tanısı bulunmamış ve psikolojik tedavi kullanmamış olan 27 sağlıklı deney kontrol grubunu oluşturmuştur. Tüm gruplardaki deneklerin depresyon ve diğer eksen I tanlarının değerlendirilmesi için DSM-IV ve DSM-IV 2011 tanımı kullanılmıştır. Depresyonun şiddeti ve etkenleri için Hamilton Depresyon Derecelendirme Ölçüğü (HAMD) kullanılmıştır. Migren tanısı için Uluslararası Baş ağrıları Birliği'nin belirlediği ölçümle karşılaştırılmıştır. Fibromiyalji tanısı için ise Amerikan College of Rheumatology ölçümle karşılaştırılmıştır. Fibromiyalji ve migren hastalarına ağılınlık ve visual analog scale (VAS) ile değerlendirilmiştir. BDNF ölçümü için serum örnekleri -70°C derecede saklanmış ve kit ile beraber verilen blok ve sample çözünümle ilâh edilerek dilüe edilmişdir. Verilerin değerlendirilmesinde, serum BDNF düzeylerinin yanısıyla etkenlerin değerlendirilmesinde Spearman sıralı korelasyon testi, cinsiyet ve etkenlerin değerlendirilmesinde Bonferroni düzeltmeli Mann Whitney U testi kullanılmıştır. Serum BDNF düzeylerini ölçen istatistiksel olarak anlamli bir fark bulunmuş ve kontrol grubunun (32.4±11.3 ng/ml) düzeyi, fibromiyalji ve migren hastalarının (30.7±8.9 ng/ml) düzeyinden anlamli bir fark bulunmaktadır. Serum BDNF düzeyleri ile yaş ve cinsiyet arasındaki istatistiksel olarak anlamli bir ilişki yoktur (r=0.097, p=0.579). 

Sonuç: Ağır sendromlar stres ile ilişkilendirilebilir, bu çalışmadan, stresin bir belirtecii olan serum BDNF düzeyi bu görüşe dayanmaktadır. Bu durumun ne denli fibromiyalji ve migren gibi ağrılı sendromlarda serum BDNF düzeylerinin periferik trofobüst iştırmelerindeki değişiklikleri etkisemesi olabilir. Bu yandan belir bir düzeyde kaletro kronik stres durumlarında serum BDNF düzeylerinin etkiliyormudur olmaz da bu durumda rol oynamaz olabilir.

Anadır sözcuları: BDNF, depresyon, fibromiyalji, migren

Klinik PsikoFarmakoloji Bülteni 2008;18:259-265

INTRODUCTION
In the recent years, several studies have suggested that intracellular pathways regulating neuroplasticity and neurodegeneration have a major role in the etiology of mood disorders (1). Neurotrophins play a critical role not only in neuronal and glial development and function, but also in cell survival, maintenance as well as the functional and structural integrity of the adult brain (2). A role for neurotrophins in neuronal plasticity has been reported and it shows that neuronal activity regulates the expression, secretion and signaling of neurotrophins to induce specific changes in synaptic efficacy and synapse morphology (3). Brain-Derived Neurotrophic Factor
(BDNF) is a member of the nerve growth factor family. It is one of the several endogenous proteins, which are important for maintenance of synaptic function and neuronal survival in addition to the plasticity in the central nervous system (CNS) (4,5). Recent findings have suggested that BDNF is involved in the pathogenesis of mental disorders, especially major depression (6,7). It is already known that serum BDNF levels are lower in patients with depression; and the levels are correlated with the severity of the disease (8,9).

A number of studies (10,11) suggest that neurotrophins are regulated in response to stress. Stress can affect the expression of BDNF itself, which has a function as a defense mechanism against stress (10–12). Recent studies have suggested a role for BDNF in depression as well as in other stress-related disorders (13,14). According to this hypothesis, depression occurs as a result of stress related to neuronal atrophy and decreased neurogenesis, and via the stimulation of intracellular pathways antidepressants lead to an up-regulation of cyclic adenosine monophosphate (cAMP) response element binding (CREB) protein and result in increased expression of neurotrophic factors, particularly BDNF (15,16). Since pain syndromes such as migraine and fibromyalgia (FM) are also related with stress and depression, and since these syndromes benefit from antidepressant treatment, it is a point of interest whether these syndromes are aggregates of depression. Furthermore, studies involving families, pain syndromes have been shown to be related to the affective disorders (17,18). Moreover, recent findings have suggested that BDNF is also involved in the pathogenesis of major depression, which is often associated with pain syndrome (19).

On the other hand, BDNF has been shown to be related to chronic pain. BDNF contributes to the structural and functional plasticity of nociceptive pathways in the CNS, within the dorsal root ganglia (DRG) and the spinal cord. Release of BDNF appears to modulate or even mediate nociceptive sensory inputs and pain hypersensitivity (20,21). In animal models, BDNF mRNA expression in hippocampus has been shown to be decreased under pain, that is similar to stress and depression (22). In contrast, rats suffering from chronic pain showed increased levels of BDNF in their frontal cortex (23).

In the present study, we aimed to compare the level of BDNF in patients with migraine and FM with that of depressive patients and healthy subjects. The hypothesis was that the serum levels of BDNF in pain syndromes are equal to the levels in major depression and lower than the levels in the healthy control group since they are also related to stress. This study is the first study to assess patients with both migraine and FM comparing with depressive patients in terms of serum BDNF levels.

MATERIAL and METHODS

This descriptive study was performed in the Department of Psychiatry of Celal Bayar University Hospital, Manisa, Turkey between February 2004 and May 2005.

Subjects

The inclusion criteria of the study were to be between 18-65 years old, to comply with the study procedure and to accept to be included to the study. The exclusion criteria of the study were to have a co-morbid diagnosis for the study groups, to receive psychiatric drug treatment in the last eight weeks, and to have any neurological diseases. Twenty-seven patients who had not received any previous antidepressant treatment and psychiatric diagnosis were involved in the migraine group, and 19 patients who had not received any previous antidepressant treatment and psychiatric diagnosis were included in the fibromyalgia (FM) group. In the depression group, 24 patients with at least eight weeks of antidepressant-free period were asked to participate in the study. All patients were planned to receive antidepressant treatment after blood sampling. All patients were informed about the study aim and procedure and so no patient was excluded during the procedure. In the major depressive disorder group, 19 (79.1%) patients were having their first episode; the other five (20.9%) patients had recurrent episodes. The mean age of onset for depressive disorder group was 33.4±12.9 years, and the mean duration time of depression in the group was 2.5±3.9 years. In the MDD group the patients did not have any comorbid diagnosis according to SCID-
I. All subjects in both depression group and pain syndrome groups were presently in the exacerbation period when they were included to the study. In the healthy control group, 26 subjects who had not received any previous psychiatric diagnosis and psychiatric treatment were invited to the study. Those who participated in the study did so voluntarily and having given their written informed consent. This study was approved by the local ethics committee.

**Psychometric Tests used**

Structured Clinical Interview for DSM-IV (SCID-I): SCID-I is a structured interview form for the assessment of DSM-IV axis I disorders and it was applied by a trained psychiatrist familiar with psychopathology. This form was developed by First and colleagues (1997) (24) and it has been adapted into Turkish by Ozkurkcugil and colleagues (1999) (25). All participants were evaluated with SCID-I which is a structured interview form aimed at diagnosing psychiatric diseases according to DSM-IV. The mean time for the interview of SCID-I was between 30 – 45 minutes.

Hamilton Depression Rating Scale (HAM-D): For the assessment of the severity of depression, the structured interview of Hamilton Depression Rating Scale (HAM-D) was used. The 17-item Hamilton Depression Rating Scale (HAM-D) with structured interview guide is used (26); and the reliability and validity study for the Turkish version was performed by Aydemir et al. (27). As the score of the HAM-D increases the severity of depression also increases. HAM-D is not used with the purpose of making diagnosis of depression.

All participants were examined by a Physical Therapy and Rehabilitation specialist and the diagnosis of FM was based on the criteria of the American College of Rheumatology (28). Patients in migraine group were evaluated by a Neurologist and the diagnosis of migraine was established according to the criteria of the International Headache Society (29).

Patients were asked to describe the severity of their pain on a visual analogue scale (VAS). Vertical and horizontal VAS was developed by Scott and Huskisson in 1979 (30). In our study vertical form of VAS (0-10cm) was used. The patient was asked to show his/her severity of pain in a 0-10 cm scale.

**BDNF Assessment**

Venous blood samples (5 ml) were drawn from patients and healthy controls and collected into anticoagulant-free tubes between 11:00 and 12:00 A.M. They were stored at room temperature for 1 h, and then for another 1 h at 48°C before sera were isolated. Samples were centrifuged at 48°C (3000 rpm, for 15 min using a refrigerated centrifuge) and the serum samples were transferred to a new set of polypropylene tubes. The serum samples were stored at -700°C for batch assessments. The BDNF assessment was performed in a period of 4 months beginning with the collection of the samples. Serum BDNF levels were measured by a solid-phase sandwich, two-site, enzyme-linked immunoassay (ELISA), using the BDNF Emax Immunoassay System reagents (Promega, Madison, WI, USA) according to the manufacturer’s instructions. In this procedure flat bottom, 96 well plates were coated with anti-BDNF monoclonal antibody to bind soluble BDNF and the plates were incubated overnight at 48°C. After washing the plates with wash buffer (Tris–HCl, pH 7.6) and blocking for nonspecific binding, the plates were incubated at 258°C for 1 h without shaking and later they were washed once. BDNF standards and samples, in duplicate, were added to the appropriate wells and the plates were incubated for 2 h at 258°C. The second specific BDNF polyclonal antibody was added and incubated for 2 h at 258°C so that the captured BDNF binds to the polyclonal antibody. After the washing, the amount of specifically bound polyclonal antibody was determined using species-specific anti-IgY antibody conjugated to horseradish peroxidase as a tertiary reagent. Unbound conjugate was removed by washing and incubation with a chromogenic substrate and stopping the reaction with 1 N hydrochloric acid, the absorbencies were measured at 450 nm using an automatic ELISA microplate reader. According to the BDNF analysis of performance, the intra-assay precisions (coefficient of variation, CV) were 8.8% at 28.6 pg/ml; 2.9% at 53.3 pg/ml; 2.2% at 286.1 pg/ml.

**Statistical Analysis**

We used frequency and percentage for nominal
variables and mean+standart deviation, median (min-max) for continuous variables as descriptive statistics. We compared groups by using chi square test and Kruskal Wallis test as appropriate. Bonferoni adjusted Mann Whitney U test was used as a post hoc test when statistical significant differences presence into four group. Spearman rank correlation analysis was performed to demonstrate the correlation between age, VAS and HAM-D, and serum BDNF level SPSS (Statistical Package for Social Sciences) was used for computation. A 2-sided p value <0.005 was considered statistically significant.

RESULTS

There was a statistically significant difference between the groups in respect of age, and the FM group was older than the other groups, whereas the other three groups were not significantly different from each other. The mean age of the patients in the FM group (47.3±9.9 years) (median:48, min:30, max:76) was higher than the mean age of the patients in the other study groups (p<0.0001), and the mean and median age of the depressive, migraine and control groups are 33.9±15.7 (median:28, min:18, max:78), 36.6±11.4 (median:37, min:18, max:61), 31.4±5.9 (median:32, min:23, max:41) years respectively. Also, there was a significant difference among the study groups in terms of gender and in the FM group, there was a female predominance (χ²=10.13, p= 0.005). (Table 1).

In pain syndromes there was not a statistically significant correlation between age and serum BDNF level (r= -0.223; p= 0.137). Also gender did not cause significant difference on the serum BDNF levels (Z= -0.108; p= 0.935). In the depression group both age (r= 0.121; p= 0.573) and gender (Z= -0.591; p= 0.555) was not significantly related to serum BDNF levels.

The mean HAM-D scores of the depressive, migraine, FM, and control groups were 21.01±3.60 (median:20, min:14, max:29), 4.92±2.31 (median:5, min:1, max:9), 5.88±1.67 (median:5, min:3, max:9) and 2.3±1.72 (median:2, min:1, max:6) respectively. The VAS score was 6.70±2.10 (median:7, min:1, max:10) in the migraine group, and 6.41±1.58 (median:6.50, min:4, max:10) in the FM group. (Table 1). In the pain syndrome group there was not a significant correlation between serum BDNF levels and VAS scores (r= 0.191; p= 0.204) and HAM-D scores (r= 0.085; p= 0.579). Also in the depression group serum BDNF level was not correlated with HAM-D scores (r= 0.122; p= 0.579).

Serum BDNF level of the depression group (mean=21.24±11.30, median=18.83 ng/ml) was statistically lower than that of the migraine group (mean=32.20±10.15, median=33.76 ng/ml), FM group (mean=30.75±8.94, median=32.00 ng/ml) and the control group (mean=31.42±8.85, median=28.84 ng/ml)

Table 1: Demographical and clinical features of the study groups.

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>Gender</th>
<th>Age (mean±SD)</th>
<th>HRSD (mean±SD)</th>
<th>VAS (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression (n=24)</td>
<td>Male= 7 Female= 17</td>
<td>33.9 ± 15.7</td>
<td>21.01 ± 3.6</td>
<td>6.70 ± 2.10</td>
</tr>
<tr>
<td>Migraine (n=27)</td>
<td>Male= 2 Female= 25</td>
<td>36.5 ± 11.4</td>
<td>4.92 ± 2.31</td>
<td>6.41 ± 1.58</td>
</tr>
<tr>
<td>Fibromyalgia (n=19)</td>
<td>Male= 0 Female= 19*</td>
<td>47.3 ± 9.9**</td>
<td>5.88 ± 1.67</td>
<td>-</td>
</tr>
<tr>
<td>Healthy Control (n=26)</td>
<td>Male= 6 Female= 20</td>
<td>31.4 ± 5.9</td>
<td>2.3 ± 1.72</td>
<td>-</td>
</tr>
</tbody>
</table>

* Female gender was dominant in the fibromyalgia group.
**The mean age was higher in the fibromyalgia group.

Table 2: The serum BDNF levels (ng/ml) of study groups

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>BDNF Levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression (n=24)</td>
<td>Mean= 21.24±11.30 Median= 18.83 Min. – Max. 8.36 – 41.93 Range 33.57</td>
</tr>
<tr>
<td>Migraine (n=27)</td>
<td>Mean= 32.20±10.15 Median= 33.76 Min. – Max. 11.15 – 50.63 Range 39.48</td>
</tr>
<tr>
<td>Fibromyalgia (n=19)</td>
<td>Mean= 30.75±8.94 Median= 32.00 Min. – Max. 13.14 – 48.14 Range 35.00</td>
</tr>
<tr>
<td>Healthy Control (n=26)</td>
<td>Mean= 31.42±8.85 Median= 28.84 Min. – Max. 22.25 – 50.97 Range 28.72</td>
</tr>
</tbody>
</table>

* Depresssion < other groups (Z= 14.234; p< 0.0001) (Kruskal Wallis).
** (BDNF: Brain-Derived Neurotrophic Factor).
The level of BDNF was not significantly different in the migraine, FM and control groups (Figure 1, Table 2).

**DISCUSSION**

When we compared serum BDNF levels of the four groups, we found that they were significantly lower in patients with depression. On the other hand, serum BDNF levels were similar in FM, migraine and healthy groups. We expected to find serum BDNF levels of pain syndromes as low as that of depression because these syndromes are all associated with stress (31). However, according to the hypothesis that painful conditions are a phenomenon related to stress, serum BDNF levels were expected to be lower than controls and similar to those of the depression group.

Fibromyalgia is characterized by widespread and chronic musculoskeletal pain, increased sensitivity to palpation, fatigue, sleep disturbance and morning stiffness (31). The etiology and pathogenesis of FM has not yet been fully understood. Patients with FM also, frequently report elevated levels of depression, anxiety and psychosocial stress (32,33) and recently there is a growing body of evidence that biological as well as psychosocial stress play a key-role in the pathogenesis of FM (31). Some studies show that FM is characterized by a pattern of HPA axis dysfunction, which is distinct from the pattern observed in post traumatic stress disorder (PTSD) (34,35). In family studies, pain syndromes are shown to be related to the affective disorders (17,18).

Migraine is characterized by an episodic occurrence. It is widely known the hypofunction of the serotonergic system in migraine (36). There is evidence from some cross-sectional and longitudinal studies performed in general population suggesting that depression and anxiety disorders are associated with migraine (37,38). It is widely known that pain syndromes such as migraine and FM are also related to stress and depression (20,31,33,37-39). From this perspective, it is rational to expect BDNF levels to be lower than controls in both conditions whether painful syndromes are substitutes for depression or they are stress related syndromes. Several previous studies have shown that BDNF expression is decreased in acute and chronic stress conditions and depression (9,13,15,16,22).

Very few studies in the literature evaluate BDNF levels in painful syndromes. In one study investigating the peripheral BDNF levels in cases of primary headache (migraine with aura, migraine without aura and cluster headache), plasma BDNF levels were found to be higher in migraine patients without aura than in controls (40). In addition, platelet BDNF levels were found to be lower than controls in patients with migraine or cluster headache in the same study. Authors interpreted these results as the potential of primary headache patients to carry polymorphisms of the genes encoding neurotrophins (BDNF and NGF), which may affect either their expression or release from the peripheral cellular sources. Moreover, they emphasized that platelet neurotrophins might be relevant biological markers for the study and clinical management of primary headache disorders, but this was not relevant for serum neutrotrophins (41). In fact, platelets are known to be activated in patients with migraine (40). Hence, we can conclude that platelets may be a major source of BDNF in serum.

In another study that evaluated serum BDNF levels in patients with FM, BDNF levels were found to be significantly higher in patients compared to healthy volunteers. Moreover, it was reported that these levels were independent from age, gender, duration of disease, preexisting recurrent major depression or anti-depressive medications received in low analgesic doses (20). Authors interpreted this finding, as it might be reasonable to assume that platelets are also activated in FM. This could explain their results that showed a
significant increase in the serum concentration of BDNF in patients with FM (20) similar to migraine patients. Although an animal study found a positive correlation between serum and cortical BDNF levels (42), the majority of BDNF in plasma and serum might be of peripheral origin as described above (20).

Furthermore in some studies it was shown that chronic sub-threshold stress does not cause any effect on serum BDNF level (43). Similarly in a study where the serum BDNF levels of dysthymic patients were higher than that of the patients with major depressive disorders and equal to that of healthy controls (44), the authors suggested that chronic stress in dysthymia does not affect serum BDNF levels. Thus chronic stress in dysthymia can possibly cause desensitization which may be associated with the tendency of BDNF to remain unchanged.

In this present work contrary to our hypothesis serum BDNF levels of FM and migraine groups was found to be higher than that of major depressive group and equal to that of healthy controls. One possible cause of this finding may be the increase of serum BDNF levels in pain syndromes due to the platelet activation. Since platelets contain high amount of BDNF, peripheral processes seem to affect serum BDNF levels (40,41). One other cause is that serum BDNF levels may not be affected by the chronic sub-threshold stress. A similar case was reported in dysthymia (44). In both cases the results of this present study indicate that serum BDNF levels alone cannot be a sufficient variable in demonstrating the relation of stress with pain syndromes such as migraine and fibromyalgia.

There were some limitations to this study. First, the number of the patients in the study groups, especially in the FM group, was too low to generalize the results. These results should be repeated in larger patient groups. Secondly, the distribution of gender among the study groups was not even. So the results should be read with that in mind. Another limitation was the measuring of BDNF in serum since it is an indirect way to put the gene expression of BDNF forward. Consequently, further studies that concomitantly address all variables including stress, neurotrophic factor, or depression severity are required. The lack of BDNF level measurements in peripheral platelets was also another limitation to this study.

In conclusion, serum BDNF levels alone cannot be a biological indicator that demonstrates the relationship between stress and pain syndromes. Although these painful conditions may not be equivalents of depression in terms of stress (contrary to data from many other studies), peripheral physiological processes (such as alterations in platelet functions) may affect serum BDNF levels, particularly in painful syndromes. We believe that the latter is more probable. Also, chronic stress seem not to affect serum BDNF levels. Serum BDNF levels may not give adequate information as a marker in painful syndromes such as other stress related disorders.

Besides, some peripheral influences may also alter serum BDNF levels. Hence, measuring serum BDNF levels alone might not give adequate information (at least for pain syndromes). Measuring BDNF levels in peripheral platelets as well as serum would be a more appropriate approach in further studies.

ACKNOWLEDGEMENT
The authors wish to thank to Wyeth Turkey who partially supported this study by financing the laboratory kits.

References:


23. Domic V, McCarson KE. Hippocampal neurokinin-1 receptor and brain-derived neurotrophic factor gene expression is decreased in rat models of pain and stress. Neuroscience 2005; 133:999-1006


28. Aydemir O. Deveci A, Icelli I. Reliability and validity of the Turkish version of the structured interview guide for Hamilton Depression Rating Scale Seasonal Affective Disorder. Psychiatry in Turkey 2006;8:18-21