VITAMIN D LEVELS AND VITAMIN D RECEPTOR GENE POLYMORPHISM IN MAJOR DEPRESSION

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SUMMARY

Background: The aim of this study is to evaluate vitamin D levels and rs2228570 (FokI) polymorphism of vitamin D in patients with established diagnosis of major depressive disorder in order to investigate the impact of vitamin D levels and genetic polymorphisms on etiology and/or severity of the disease.

Subjects and methods: The study included 86 patients who were diagnosed with major depressive disorder in Hospital of Balıkesir University Faculty of Medicine, Department of Psychiatry, and 89 healthy volunteers with similar age, sex, education level and BMI. Psychiatric diagnosis was established by using Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). For clinical evaluation, sociodemographic data form, Hamilton Depression Rating Scale, Hamilton Anxiety Scale were used. Blood samples were drawn after 12 hours of fasting from the patients volunteered and the control group who were given their informed consent for participation in the study. Vitamin D levels were determined by using the method of ECLIA (Electrochemiluminescent immunoassay). Genotype analysis was performed using the method of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

Results: In our study, median vitamin D levels (min-max) of the patient and control groups were 10.3 ng/mL (3.0-42.1) and 11.4 ng/mL (3.0-38.8), respectively. Statistically significant differences as for vitamin D levels between groups were not detected (p=0.729). Similarly no statistically significant difference between groups in genotype distribution was observed (p=0.396).

Conclusion: In conclusion, our findings do not support the relationship between depression, vitamin D levels and Fok 1 polymorphism of vitamin D receptor. To test these hypotheses in the light of literature we need further studies to be performed with large number of patients.

Key words: major depressive disorder (MDD) - vitamin D - VDR

INTRODUCTION

Major depressive disorder (MDD) is a mental disorder which causes serious functional loss lasting for at least two weeks, and characterized by depressed mood that is accompanied by loss of interest, pleasure, and depressive mood (Birliği 2013). The monoamine hypothesis, effect of stress, structural changes in the brain, the hypothalamus-pituitary axis abnormalities and neuroendocrine changes such as hypothyroidism have been studied as its pathophysiologic factors.

Vitamin D is a steroid hormone known for a long time for the effect on homeostasis of calcium and phosphorus. Its active metabolite 1.25 (OH) 2D has both genomic and non-genomic effects. Besides our well established knowledge about its effects on bone metabolism, presence of vitamin D receptor (VDR) and 1 alpha- hydroxylase enzyme, which converts 25 (OH) D to 1.25 (OH) D², in the brain, has launched the explanation efforts of the relationship between brain development and neuropsychiatric disorders (Garcion et al. 2002). VDR has been identified in many tissues, including neurons and glial cells, and particularly prefrontal cortex, hippocampus, cingulate gyri, thalamus, and hypothalamus (Eyles et al. 2005). In addition, the mostly known active form of vitamin D, 1.25 (OH) 2 D3, has been shown in cerebrospinal fluid (CSF) (Balabanova et al. 1984). Association of these areas with depression suggests the role of vitamin D in the pathophysiology of depression.

In recent studies, it was reported that vitamin D preserves the structure and concentration of neurons via detoxification mechanisms and regulating the synthesis of neurotrophins (Neveu et al. 1994a, Neveu et al. 1994b). Detoxification mechanisms includes inhibition of the inducible nitric oxide synthase synthesis and increases in glutathione levels (Garcion et al. 2002). In rats vitamin D has been shown to have protective effects against serotonin and dopamin depleting effects of neurotoxic doses of methamphetamine (Cass et al. 2006). In addition it has been reported that vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells (Puchacz et al. 1996). This enzyme plays a role in the biosynthesis of catecholamine as a rate-limiting enzyme.

Some studies have demonstrated that 25 (OH) D which is thought to be a more reliable marker in the determination of vitamin D levels, is at a lower concent-
Sociodemographic data Form

Sociodemographic data form developed by us consists of the questions about socio-demographic characteristics such as age, sex, marital status, occupation and education level and the clinical features such as history of psychiatric disorders, alcohol, cigarette and drug use and menopausal status for women.

Structured Interview Form for DSM-IVTR Axis 1 Disorders (SCID 1/Clinical Version)

DSM-IV Axis I Disorders (SCID-I) is a structured scale consisting of 6 modules used to determine DSM-IV Axis I disorders applied by the interviewer. It was developed by First et al in 1997 and Özkürkçügil et al have done reliability and validity studies of its Turkish version (First 1997, Özkürkçügil et al. 1999).

Hamilton Depression Scale (HAM-D)

It was published by Max Hamilton in 1960, and it is still widely used to measure the severity of depression. It contains 17 items which questions symptoms of depression within the previous week. Akdemir et al have done the Turkish version reliability and validity studies of its Turkish version (Akdemir et al. 1996, M 1960).

Hamilton Anxiety Rating Scale (HAM-A)

Hamilton Anxiety Rating Scale (HAM-A) is a semi-structured question developed by Hamilton in the year 1959 to rate the severity of anxiety. It consists of 14 items which evaluate somatic, and psychic symptoms of anxiety. It was originally published by Max Hamilton in 1959 and Turkish version’ reliability and validity study has been done by Yazıcı et al (Hamilton 1959, Yazıcı 1998).

Statistical Analysis

All statistical analyzes were performed using SPSS version 15.0 and Visual graphics (histogram, etc.) and appropriate statistical tests (Kolmogorov-Smirnov and Shapiro-Wilk) were used for evaluating normality of data. Student's t-test or Mann-Whitney U test was used for the comparison of independent two group means considering the normality of distribution. For assessing categorical data in cross- tables chi-square or Fisher's exact tests were used. Scale scores and the distribution of correlations between vitamin D levels were evaluated using Pearson or Spearman test. The effect of other independent variables on the possible role of genotype and D levels on the risk of depression was examined using logistic regression analysis and adjusted OR values were calculated. In all statistical analysis, level of statistical significance was defined as p value of less than 0.05.
RESULTS

When sociodemographic data of the patient, and the control groups were compared as for sociodemographic data, age, gender, BMI, smoking, and alcohol use, any statistically significant intergroup difference was not seen (Table 1).

Besides any statistically significant difference was not observed between education levels of the participants in the patient, and control groups. In both groups married participants were more numerous, but without any statistically significant intergroup difference between groups (p=0.376).

Clinical Evaluation Scales

The median (min-max) value of HAM-D scores was 21 (10-32) for patients and 2 (0-13) for the control group. The difference between the 2 groups was statistically, and highly significant (p<0.001). The median (min-max) value of HAM-A scores was 16 (1-31) for patients and 3 (0-15) for the control group. Similarly, the difference between the 2 groups was statistically, and highly significant (p<0.001) for HAM-A scores. A strong positive correlation was found between HAM-A and HAM-D scores (r=0.864, p<0.001) (Table 2).

Vitamin D levels

Vitamin D levels median (min-max) values of the patient and the control groups were 10.3 ng/mL (3.0-42.1) and 11.4 ng/mL (3.0-38.8) respectively (Figure 1). Statistically significant difference was not detected between the groups as for vitamin D levels (p=0.729).

When vitamin D levels were evaluated in 82% of the control group and in 77.9% of patients vitamin D levels were less than 20 ng/mL which is considered the reference value for vitamin D insufficiency (p=0.496) (Risteli 2012). Still a statistically significant difference was not seen between the patient, and the control groups regarding vitamin D insufficiency.

When we evaluated the relationship between levels of vitamin D and clinical rating scales, there was no correlation between vitamin D levels and scale scores. Median (min-max) value of vitamin D levels of the patients who experienced their first episode of depression was 11.2 (3.0-38.8), 9.9 (3.4-42.2) in patients who suffered from recurrent attacks and 11.4 (3.0-38.7) in the control group. Statistically significant difference was not detected between frequency of attacks and vitamin D levels (p=0.546) (Figure 1).

Table 1. Comparison of sociodemographic data

<table>
<thead>
<tr>
<th>Age (Mean± SD years)</th>
<th>MDD (n=86)</th>
<th>Control (n=89)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>37.8±12.5</td>
<td>37.3±12.2</td>
<td>0.774</td>
</tr>
<tr>
<td>Men</td>
<td>37.6±12.4</td>
<td>39.9±11.7</td>
<td>0.664</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>26.6±7.2</td>
<td>24.9±3.9</td>
<td>0.065</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>n=75</td>
<td>n=74</td>
<td>0.45</td>
</tr>
<tr>
<td>Male</td>
<td>n=11</td>
<td>n=15</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>n=24</td>
<td>n=19</td>
<td>0.314</td>
</tr>
<tr>
<td>Regular alcohol user</td>
<td>n=5</td>
<td>n=2</td>
<td>0.272</td>
</tr>
</tbody>
</table>

Table 2. HAM-D and HAM-A scores of the study participants

<table>
<thead>
<tr>
<th>HAM-D</th>
<th>Median (min-max)</th>
<th>Control Median (min-max)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD</td>
<td>21 (10-32)</td>
<td>2 (0-13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HAM-A</td>
<td>16 (1-31)</td>
<td>3 (0-15)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3. FokI polymorphism genotype distribution

<table>
<thead>
<tr>
<th>VDR gene</th>
<th>Patient (n=86)</th>
<th>Control (n=89)</th>
<th>OR (95% GA)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF</td>
<td>32 (43.8%)</td>
<td>41 (56.2%)</td>
<td>0.694&lt;sup&gt;a&lt;/sup&gt; (0.38-1.27)</td>
<td>0.236&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ff</td>
<td>46 (51.7%)</td>
<td>43 (48.3%)</td>
<td>1.23&lt;sup&gt;b&lt;/sup&gt; (0.68-2.23)</td>
<td>0.494&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ff</td>
<td>8 (61.5%)</td>
<td>5 (38.5%)</td>
<td>1.723&lt;sup&gt;c&lt;/sup&gt; (0.54-5.50)</td>
<td>0.358&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>FF<>Ff+ff;  <sup>b</sup>Ff <> FF+ff;  <sup>c</sup>ff<>FF+Ff
In our study, we found no statistically significant difference between groups as for FokI polymorphism genotype distribution (p=0.396). While 90.7% of patients and 94.4% of control group had F allele while frequency of f alleles had been seen in 62.8% of the patients and 53.9% of the control subjects (Table 3).

**DISCUSSION**

Vitamin D deficiency has been seen in many people worldwide so it is defined as a global problem (Bhimani 2012). In Saudi Arabia, United Arab Emirates, Australia and India vitamin D levels were reported to be below 20 ng/ml in 30-50 % in children and adulthood (Fuleihan et al. 2001, Marwaha et al. 2005, McGrath et al. 2001, Sedrani 1984).

In recent years the relationship between vitamin D levels and many diseases have been frequently discussed. There is increasing number of studies trying to explain mainly the relationship between vitamin D, psychotic disorders and major depressive disorders in psychiatry. Higher incidence of schizophrenia among individuals born in the winter and spring months, increase in disease incidence in cold climates and the relationship between maternal nutritional deficiency and schizophrenia suggest the relationship between schizophrenia and low vitamin D levels (McGrath 1999). In Northern Finland, a follow-up study with 9114 participants showed that vitamin D supplementation during the first year of life is associated with a reduction in the incidence schizophrenia in men (McGrath et al. 2004). In a small scale cohort study in third trimester maternal 25 (OH)D levels were analyzed, the study participants were followed up until the age of 30 and the investigators reported that levels of maternal vitamin D may be associated with an increased risk of schizophrenia (29 vs 46%) (Eyles et al. 2003). Similarly, Zeng and colleagues also evaluated 52 patients and found that 28.8% of the patients in the study had low vitamin D levels and no difference had been seen between major psychiatric diagnoses (Zeng et al. 2016).

Studies on depression have yielded different results. As is known, depression demonstrates seasonal specialties, especially in the winter months with phototherapy improved treatment responses are achieved (Rosenthal et al. 1988). In cases of low vitamin D levels, parathyroid hormone (PTH) levels increase and hyperparathyroidism has been reported often with depressive symptoms (Özên & Haspolat 2003). JoBorn et al. mentioned that in primary hyperparathyroidism, and mild hypercalcemia, depression, anxiety and cognitive complaints are frequently seen, and one year after the parathyroid surgery, improvements in psychiatric symptoms were observed (Jobom et al. 1989). McCue et al evaluated 107 psychiatric patients and found vitamin D levels below 20 ng/ml in % 52.3 of the patients while they hadn’t observed any association between psychiatric diagnosis with vitamin D levels (McCue et al. 2012). Vitamin D deficiency has been associated with deterioration in cognitive function and depressive mood particularly in elder age (Hoogendijk et al. 2008, Wilkins et al. 2006). Milaneschi and his colleagues found that low baseline vitamin D is associated with an increased probability in developing depressive mood after 6 years of follow-up in people above 65 years of age. Especially in women this relationship between vitamin D levels, and depression stronger (Milaneschi et al. 2010). In another study Schneider et al. compared 34 schizophrenic, 25 depressive and 30 alcohol-dependent patients with healthy controls, and lower levels of 25 (OH) D and 1.25 (OH) D were found in patients. The authors thought that these lower levels might be associated with low socioeconomic status and unhealthy eating habits (Schneider et al. 2000). In a large population study carried out by Ganji and colleagues people who had active depressive symptoms had lower vitamin D levels than those without with an incidence rate of 8.4% (Ganji et al. 2010). In 2014 Yilmaz et al found negative correlation between vitamin D levels and Beck Depression Inventory scores in premenopausal women (Yilmaz et al. 2014). However, similar to our study , in 2010, Zhao et al reported lack of any relationship between vitamin D levels and major or minor depressive disorders (Zhao et al. 2010).

There are many studies concerning vitamin D supplements in depression with inconsistent results. Landonwne and Provost provided vitamin D support in winter months to healthy people and indicated an increase in positive affect scores (Landsdowne & Provost 1998). Dumville and his colleagues reported that during winter months, receiving daily doses of 800 IU vitamin D made no changes in mental well-being in women over 70 years of age (Dumville et al. 2006). Also, Haris and his colleagues reported the results of 1 year of vitamin D supplementation to 125 women with seasonal affect swings had not exerted positive effects on these changes (Harris & Dawson-Hughes 1993). Khoraminya et al treated 21 major depressive disorder patients, with 20 mg of fluoxetine and 1500 IU of vitamin D combination for 8 weeks and 21 patients only with fluoxetine in a double-blind placebo-controlled randomized trial. They indicated that combination therapy was superior to fluoxetine treatment after 4 weeks of study (Khoraminya et al. 2013). In 2014 Spedding reported that vitamin D supplementation provided statistically significant improvement in depression after excluding biological flaws in a meta-analysis of 15 randomized controlled trials. Biological flaws were listed as, using inappropriate supplements, decrease in vitamin D levels after vitamin supplementation, taking ineffective doses of vitamin D, failure to measure baseline levels of vitamin D, and in cases with deficient levels of vitamin D (Spedding 2014).

The results of polymorphism analysis are different in each community so it is adequate to calculate VDR genotype frequencies for each population. The only VDR polymorphism that changes the structure of receptor is a polymorphism in exon 2 and Fok I restriction
enzyme is used for its determination (Uitterlinden et al. 2004). In Turkey, in a study of 100 healthy individuals realized in the year 2002, VDR alleles (F: 0.73, f: 0.27) and VDR genotype frequencies (FF: 55%, Ff: 36%, FF: 9%) were detected, as indicated (Dayanąç 2002). In our study, we found similar results, however any difference was not observed in genotype distribution between patients and controls.

Experimentally, VDR mutant mice showed deteriorated social behavior, increased anxiety-like behavior and abnormal grooming. But Kalueff et al could not determine depression-like behaviour in these mice (Kalueff et al. 2004). In human studies; Yan and his colleagues reported lack of any relationship between VDR variants and schizophrenia in DNA analysis of 192 patients (Yan et al. 2005). Ahmadi et al reported that Ff genotype was more frequent in patients with bipolar disorder and is associated with decreased in D1 dopamine receptor gene expression (Ahmadi et al. 2012). In another study, Kuningas reported that individuals over 85 years of age who had BsmI and TaqI polymorphisms were more sensitive to age-related cognitive deterioration, while the Apal polymorphism was protective against the effect of deterioration and depressive symptoms (Kuningas et al. 2009). Similar to our study, in their study any relationship was not observed between depressive symptoms and FokI polymorphism. Also Özdolap and colleagues could not find any significant differences between patients with fibromyalgia which is often accompanied by depression and control group regarding both vitamin levels and allele frequencies (Özdolap 2012).

Our study has some limitations, and strengths as well. Measurement of 25 (OH) D as the best indicator of vitamin D level, diagnosis of depression by clinician and applying clinical scores by experienced physicians, exclusion of confounding factors that may affect vitamin D levels such as age, physical activity, chronic illness, cancer, cardiovascular system pathologies, autoimmune diseases are the strengths of our study. Although the incidence of depression is higher in women in the community, since number of female participants in our study is more than three times the number of men so it is difficult to generalize results to general population.

**CONCLUSION**

Our results do not support the hypothesis that vitamin D levels and FokI polymorphism of VDR gene play a role in the etiology of depressive disorder. We think that that relationship between depression and vitamin D deficiency is not a cause-effect relationship, but two events seem to trigger each other. Some lifestyle changes, such as reduced food intake, or reduced time of sun exposure because of psychomotor retardation in depression may lead to vitamin D deficiency. There is evidence in literature that vitamin D enhances positive mood. However, for precise determination of this phenomenon, placebo-controlled studies should be performed where priorly baseline vitamin D levels of the patients followed up with major depressive disorder should be measured, and given vitamin D supplements to optimize their vitamin D levels.

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**Conflict of interest:** None to declare.

**Contribution of individual authors:**

Merve Şahin Can & Hayriye Baykan participated in the design of the study.

All authors participate in literature search and analyses, interpretation of data and writing of the manuscript.

**References**


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