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Original Article

The Effects of Vitamin D on Kynurenine Level in Children With Attention Deficit Hyperactivity Disorder: An Epidemiological Study

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Abstract

Background and aims: Attention deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders with a worldwide prevalence of 5%. The prevalence is even higher among school age children ranging from 8% to 12%. ADHD is predominantly childhood-onset disorder and can persist into adolescence and adulthood to inflict long-term harm. The aim of this study was to identify the epidemiological features of ADHD and investigate the effects of vitamin D on kynurenine level in children with ADHD in Iran using ELISA method.

Methods: A case-control study was designed. The study participants consisted of 40 patients with ADHD and 40 healthy participants as control group. It was conducted in Isfahan Hasht Behesht hospital from July to November 2015. All samples were treated with 1000 U of vitamin D as a tablet twice daily. The level of kynurenine was measured in blood samples using enzyme-linked immunosorbent assay (ELISA) method. Data analysis was done using SPSS software.

Results: The results of this study showed that the mean vitamin D levels were 49.73 and 73.72, respectively before and after treatment in patient group, which seem significant. The mean vitamin D level after treatment was higher in patient group in comparison with control group. On the other hand, the mean kynurenine levels were 608.1 and 662.9, respectively before and after treatment in patient group. The mean kynurenine level was the same before and after treatment.

Conclusion: According to the results of the study, we can conclude that vitamin D did not have a significant effect on kynurenine level in children with ADHD.

Keywords: Attention deficit hyperactivity disorder, Vitamin D, Kynurenine, ELISA.

Introduction

Regarding the psychological basis of human development, childhood is an important life stage during which psychiatric disorders may also occur. Attention deficit hyperactivity disorder (ADHD) is a developmental disorder which is described by impulsiveness, inattention and hyperactivity,[1] known as one of the most common psychiatric disorders in children and teenagers.[2] According to the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V), there are 3 types of ADHD. These subtypes of ADHD consist of inattentive (ADHD-I), predominately hyperactive-impulsive (ADHD-HI) and combined (ADHD-C), among which the inattentive and combined subtypes are the most prevalent.[3] Some impulsive, inattentive or hyperactive symptoms display before the age of 7 years and may cause impairment in this period of life.[2] Although the etiology of ADHD is not known, it is explained as a developmental failure in the brain mechanism in which inhibition is involved and underlies self-control.[4]

The prevalence of ADHD from different geographical regions worldwide was reported as 5.3% in childhood and estimated as 1% to 4% in adulthood.[5] Studies indicate that boys are affected more frequently than girls, with the ratio ranging from 3:1 to 9:1.[6] Family, twin and adoption studies have shown an extreme genetic component in

susceptibility to ADHD. [4] Some twin studies have estimated the heritability of ADHD to range from 39% to 91% for various symptoms of the disorder.[7,8] Genetic and environmental factors are known to be involved in the etiology of ADHD.[9,10] Therefore, related genetic studies have proposed candidate genes associated with the etiology of ADHD, such as genes involved in serotoninergic and dopaminergic pathways, as well as genes involved in neuroplasticity and neurotransmission.[11] Epidemiological studies in Iran demonstrated that 15% of preschool children had ADHD symptoms.[12] Another study revealed that the prevalence of ADHD in preschool-age children in northeastern Iran was 12.3% (95% CI: 10.3–14.2%).[13] A study showed that the heritability of ADHD in female and male adults was estimated around 30%.[14]

Evidence of the presence of vitamin D receptors in the fetal and adult brain in humans has highlighted the potential impact of vitamin D on the development of mental and psychiatric disorders.[15] This vitamin is not only involved in bone metabolism and serum calcium regulation but also has significant effect on many body organs.[16] Vitamin D acting early in life on the developing brain has been related to healthier cognitive and mental phenotypes. In addition, studies conducted in children and adolescents have shown lower vitamin D levels in ADHD children compared with children without the disorder.[17] However, a large prospective study found no association of vitamin D status at 9 years of age with hyperactivity–inattention problems at 11 years of age.[18] For tryptophan, kynurenine and their metabolites the analyses were extracted from samples and calibrators/controls. About 80% to 95% of brain L-tryptophan are metabolized in the kynurenine pathway with the remainder contributing to the synthesis of 5-HT.[19]

In this study we sought to identify the epidemiological features of ADHD and compare effects of vitamin D on kynurenines serum levels among Iranian ADHD patients (n = 40) and control group (n = 40) using enzyme-linked immunosorbent assay (ELISA) technique.

Methods

Patient and Control Population

A total of 40 children with ADHD and 40 controls were recruited and phenotypically characterized by a team of experienced psychiatrists. All samples were collected from July to November 2015 from southwest of Iran. All the participants' parents agreed to participate in the study and written informed consent was obtained from all participants. The patient index was required to be between 7 and 12 years. The mean age of the affected children was 9.5 years. All children in the case group were treated with 1000 U of vitamin D as a tablet. After obtaining informed consent, 5 mL blood samples were taken from donors and collected in EDTA-containing tubes. The blood samples were transported to the laboratory and stored at -20°C until needed for analysis. The vitamin D tablets were taken twice daily, at breakfast and bedtime. Each tablet contained 1000 U of vitamin D. A full 2-month supply of vitamin D tablets was provided to each subject by the pharmacist.

ELISA Technique

The concentrations of the kynurenine in peripheral blood of treated patients were evaluated by ELISA technique using commercially available kits (Bioassay technology, Germany). The assay was performed according to the manufacturer's instructions and the plates were read at 450 nm. The sensitivity of the kit was 0.027 ng/L and all values below the sensitivity of the kits were considered as absent or zero.

Statistical Analysis

Comparisons between the kynurenine level in cases and controls were performed using the standard Pearson Q-square test, student's *t* test, Mann-Whitney U, Spearman and Pearson correlations, Kruskal-Wallis test and one-way analysis of variance (ANOVA). The assumption of normality was investigated by Kolmogorov-Smirnov test. Independent student's *t* test and Mann-Whitney U test were used for quantification analysis of ELISA test. Statistical Package for the Social Sciences (SPSS) software, version 24.0 (SPSS Inc. Chicago, IL, USA) was used for analysis and *P* values less than 0.05 and odds ratio (OR) with 95% CI were considered as statistically significant

Results

Eighty participants were included in this study, 40 ADHD patients and 40 controls. Kolmogorov-Smirnov test was done for all samples to test the normality of the data. The results of Kolmogorov-Smirnov test is shown in Table 1. In this test, significance level for the variable amount of vitamin D was obtained as 0.788 and 0.441 before and after treatment, respectively.

As the results of this study showed, the mean vitamin D levels before and after treatment were 49.73 and 73.72, respectively that seems significant. The mean vitamin D level was greater after treatment compared to the control group (Table 2).

On the other hand, the mean kynurenine levels

Table 1. The Results of Kolmogorov–Smirnov Test in Healthy and Patient Groups

		Before n = 40	After n = 40
Normal Parameters[a,b]	Mean	49.7375	73.7275
	SD	20.03051	23.70818
Most Extreme Differences	Absolute	0.103	0.137
	Positive	0.094	0.137
	Negative	-0.103	-0.104
Kolmogorov-Smirnov Z		0.652	0.866
Asymp. Sig. (2-tailed)		0.788	0.441

[a] Test distribution is normal; [b] Calculated from data.

Table 2. The Results of Vitamin D Descriptive Indicators in Healthy and Patient Groups

Paired Samples Statistics					
		Mean	n	SD	SEM
Pair 1	Before	49.7375	40	20.03051	3.16710
	After	73.7275	40	23.70818	3.74859

Abbreviation: SEM, standard error of the mean; SD, standard deviation.

Table 3. The Results of Kynurenine Descriptive Indicators in Healthy and Patient groups

Paired Samples Statistics					
		Mean	n	SD	SEM
Pair 1	Before	608.1250	36	194.94736	32.49123
	After	662.9389	36	299.93428	49.98905

Abbreviation: SEM, standard error of the mean; SD, standard deviation.

before and after treatment were 608.1 and 662.9, respectively. The mean kynurenine level was the same before and after treatment, compared to the control group (Table 3).

Discussion

ADHD is a highly heritable disorder in childhood with negative lifetime and significant functional impairment outcomes across all developmental stages. The disorder is marked by disruption of catecholamine signaling, with mainstay treatments for the disorder targeting the dopamine and noradrenaline transporters and the alpha 2A-adrenoceptor.[20] ADHD affects approximately 3% to 6% of children and adolescents from different geographical regions worldwide with a high social and educational morbidity.[21] Previous epidemiologic cross-sectional studies conducted in children have shown lower circulating concentrations of vitamin D in children with ADHD than in children without the disorder.[22] Vitamin D takes part in important processes of brain development including neuronal differentiation, regulation of reactive oxygen species and neurotropic factors, and gene expression of mitochondrial, cytoskeletal, and synaptic proteins.[23] Antioxidant and anti-inflammatory properties of vitamin D could be of key importance for protecting the developing brain against harmful environmental triggers of human psychiatric disorders including ADHD.[24] In this study, we evaluated the effects of vitamin D on kynurenines serum levels in ADHD patients in comparison with normal samples. The results of present study indicated the mean vitamin D level was higher after treatment and they were 49.73 and 73.72, respectively. However, the mean kynurenine levels were 608.1 and 662.9, respectively before and after treatment.

Although few studies have implicated the relationship between ADHD and vitamin D, extensive studies have investigated the role of this vitamin in other psychiatric/neurologic disorders including Alzheimer disease depression, schizophrenia and autism.[21] The finding of a report in Turkey among 7 to 18 years old children where a significant difference ($P<0.05$) in mean serum vitamin D level between cases (20.9 ± 19.4 ng/mL) and control groups (34.9 ± 15.4 ng/mL) was demonstrated.[17] Another study on 1331 cases of ADHD and the same number of healthy individuals in control groups under the age of 18 found that the mean serum vitamin D level in ADHD children (16.6 ± 7.8 ng/mL) was lower compared with the control group (23.5 ± 9.9 ng/mL). In addition, 8.15% of the cases with ADHD had normal vitamin levels in their serum.[22] In an interventional study on 80 patients with ADHD above the age of 16 in New Zealand, 27% prevalence rate of vitamin D deficiency was reported. Using vitamin D supplement for eight weeks was found to be effective in alleviating the signs of the disease. However, adding other micronutrients such as zinc, vitamin B12, iron and folate was not found effective.[25] In contrast, another study in England reported no significant association between some behavioral problems including ADHD and vitamin D level.[18]

The essential amino acid tryptophan is catabolized mainly through the kynurenine pathway. Altered circulating levels of kynurenines have been reported in chronic inflammatory conditions and in several neuropsychiatric disorders, including depression and schizophrenia. Candidate gene studies suggest that genes related to the kynurenine catabolism may be associated with ADHD. Additionally, ADHD patients often report comorbid depression or anxiety. In a recent study on ADHD and its relation to low birth weight, it was suggested that genetic variants in the kynurenine

pathway might contribute to ADHD symptom severity.[26] Increased catabolism of tryptophan to kynurenine has been found in patients with depression, schizophrenia, women with postpartum depression and women with preeclampsia.[27]

Our results suggest that there may be differences between ADHD patients and controls in vitamin D level. Differences in kynurenine level between ADHD patients and controls can also be seen. Furthermore, according to the results of the study we can conclude that vitamin D did not have a significant effect on kynurenine level in Iranian children with ADHD.

Ethical Approval

The experiment protocols and informed consent forms were approved by the Human Studies Committee at the Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Conflict of Interest Disclosures

The authors declared no conflicts of interest.

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