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# Comparative study of vitamin D levels in children with autism spectrum disorder and normal children: A case-control study

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## *Abstract*

**Introduction:** Autism Spectrum Disorder (ASD) is a group of neuro-developmental disorders with a genetic/environmental origin. Recently, vitamin D deficiency is considered as a possible risk factor in ASD development. This study aims to compare the vitamin D serum level in children with and without autism spectrum disorder.

**Materials and Methods:** In this case-control study, 13 children (3 to 12 years old) referred to the outpatient clinic of Ibn-e-Sina psychiatric hospital in Mashhad in 2014 who received the diagnosis of ASD based on clinical interview according to DSM-IV by child and adolescent psychiatrist and score ≥30 in Childhood Autism Rating Scale were selected as the case group. Other axis I psychiatric disorders were ruled out. These children were divided into mild, moderate, and severe groups. 14 normal children without any medical or psychiatric disorders who were matched in age and sex with ASD children were selected as control group. Vitamin D serum levels were measured in the two groups through ELIZA technique. Data were analyzed by SPSS software, through descriptive statistics, Kolmogrov-Smirnov, Mann-Whitney and Pearson tests.

**Results:** The mean vitamin D level in case group was 13.00 (distance between quartile: 9.6-19.5) and in control group was 12.00 (distance between quartile: 4.9-13.2). The difference between the two groups was not significant ($P$=0.350). Also, vitamin D serum levels had no significant relationship with the severity of ASD ($P$=0.534).

**Conclusion:** Based on the results, the serum levels of vitamin D in children with and without ASD have not any significant difference and the level of this vitamin has not significant relation with the severity of ASD.

**Keywords:** Autism spectrum disorder, Deficiency, Vitamin D

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## Introduction

Autism Spectrum Disorder (ASD) is a group of neuro-developmental disorders that they may be indicated in early childhood if they have a prenatal origin or severe impairment. The symptoms of disorder are in a wide range of socio-communication problems with repetitive behaviors or patient's senses abnormalities. ASD usually relates with other neuro-behavior-cognitive disorders that these disorders almost include intelligent disabilities, speech problems, attention deficit hyperactivity or seizure (1).

Although the genetic origin of these disorders has been indicated but the interactions of environmental factors with genetic factors have increasingly gained more significance (2,3).

Recently vitamin D deficiency during pregnancy and/or early childhood is concerned as a possible risk factor in ASD development (4,5).

This deficiency due to the progression of urban life, obesity and recommendations about protection against sunlight has shown an increasing trend from 1980s (5-8).

This vitamin has a unique role in brain hemostats, embryogenesis, neuro-immunity, antioxidant and anti-apoptose effects, neuro-differentiation and gene regulation (9-11).

It should be concerned that vitamin D is not a true vitamin but it is a steroid that it produced by a chemical cascade and the beginning point of this process is the skin exposure to ultraviolet sunlight. This procedure is related with cholesterol and the final active form of vitamin-calciteriol (1,25 OH2 D) is developed (12,13).

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It is shown that last reaction of hydroxylation in production of this active form also occurs in lymphocytes and brain microglia (14).

According to the role of this vitamin in normal brain function it seems that classification of vitamin D in neuro-steroids should be evaluated. In addition, presentation of the receptors of this vitamin in neurodevelopment phases from early stages of pregnancy and increase of these receptors with age of pregnancy, suggests the role of this vitamin in neurodevelopment (15).

Based on the researches, the prenatal deficiency of vitamin D has been suggested as a risk factor in neurodevelopment disorders such as schizophrenia and autism (5,16-20).

Some of studies indicated that the level of active forms of this vitamin has reduced in autistic children so this suggests the role of this vitamin in autism disorder (18,21).

In addition, in the past decade researchers have concerned to the relation between vitamin D and neuro-psychiatric disorders to conduct more researches (5,18,21-30).

According to the increasing prevalence of autism disorder and the results of researches about the role of vitamin D deficiency in this disorder during pregnancy and childhood, treatment methods for replacement of adequate amounts of this vitamin and lack of the same studies in our country, this study aimed to assess and compare the serum level of vitamin D among children with ASD and normal children (5,31,32).

## Materials and Methods

In this case-control study that been conducted with approval of Mashhad University of Medical Sciences in 2014, 13 children (3-12 years) referred to the outpateint clinic of Ibn-e-Sina psychiatric hospital-Mashhad who received diagnosis of ASD (autism, Asperger syndrome, Pervasive Developmental Disorder Not Otherwise Specified with (roll-out of other axis I psychiatric disorders) based on clinical intervention according to DSM-IV by child and adolescent psychiatrist and score ≥30 in Childhood Autism Rating Scale selected as case group. These children divided into mild-moderate and severe groups. 14 normal children without any medical or psychiatric disorder and active microbial infection during 2 past months who matched in age and sex with ASD children, selected as control group. The serum levels of vitamin D measured in two groups through ELIZA technique and data analyzed by SPSS software, descriptive statistics, Kolmogrov-Smirnov test, Mann-Whitney and Pearson tests.

Because of ethical considerations, parents or legal guardians of both groups fulfilled the written consent form for participation in this research. Samples were ensured that all information will remain confidential and any individual or group will not allowed to using them without further consent.

Absence of any present or prior organic disease was assessed by physician in both groups because of any interaction with the results. Then, the evaluation of serum levels of vitamin D conducted through ELIZA technique in case and control groups.

Research instrument

*A)Childhood Autism Rating Scale (CARS):* This is a behavioral measurement scale that it was designed by Schopler et al. to differentiate between autism and other delayed developmental disorders such as mental disability. This scale evaluates 15 domains and every question receives 1-4 scores that every score suggests normal situation for age, mild disorder, moderate disorder and severe disorder, respectively. The scores of 1.5, 2.5 and 3.5 also use for scoring. The global score varies from 15 to 60 and the minimum score for autism diagnosis is 30. Children who receive the score of higher than 36 or they receive the score of higher than 3 in at least 5 domains, been concerned as severe autistic group. The internal consistency of scale is high ($\alpha$=0.94) and its inter-reliability between studies is 0.71 but the difference of its validity in combination with parents interview or direct observation was not significant (r=0.83). This scale was used on 606 ASD children so it was introduced as a standard instrument (33). Also, this instrument has a strong agreement with the criteria of DSM-IV and the level of this agreement was reported 88% in assessment of 274 autistic children (34).

Data analyzed by SPSS software, descriptive statistics, Kolmogrov-Smirnov test, Mann-Whitney and Pearson tests.

## Results

The case group consisted of 13 children that 84.6% of them were boys (11 patients) and 15.4% of them were girls (2 patients). The control group consisted of 14 normal children that 85.7% and 14.7% of them were boys and girls respectively (12 persons, 2 persons). The mean and standard deviation of age in both groups were 5.7 ± 2.24 years. The cases were divided into severe and moderate-mild groups based on the severity of disorder. Of 13 patients, 9 patients (69.2%) had severe disorder and 4 of them (30.8%) had moderate-mild disorder. Because of non-normal distribution of the variable of vitamin D, non-parametric Mann-Whitney test was used to compare of this variable between case and control groups.

The middle of vitamin D in patient group was 13.00 (distance between quartile: 9.6-19.5) and this variable in control group was 12.00 (distance between quartile: 4.9-13.2) but the difference between two groups was not significant (*P*=0.350) (Table 1).

**Table 1.** The comparison of middle, quartiles, minimum and maximum of vitamin D in children with and without autism spectrum disorder

| Vitamin D | Number | First quartile | Middle | Third quartile | Minimum | Maximum |
|---|---|---|---|---|---|---|
| Case group | 13 | 9.6 | 13.0 | 19.5 | 5.1 | 29.0 |
| Control group | 1 | 4.9 | 12.0 | 13.2 | 3.8 | 29.0 |
| Total | 27 | 9.0 | 12.5 | 15.0 | 3.8 | 29.0 |
| Result | P=0.350 | | | | | |

Pearson test was used to assessment the effect of vitamin D on CARS scores in patient group. The result of this test showed that CARS score has not significant relation with vitamin D level (correlation: 0.190, *P*=0.534).

## Discussion

Based on the present results, the serum levels of vitamin D in children with and without ASD have not any significant difference and the level of this vitamin has not significant relation with the severity of ASD.

Mostafa et al. evaluated the serum levels of 25 hydroxy vitamin D and anti macrophage autoantibody in 50 autistic children in range of 5-12 years and 30 matched normal children. They found that the autistic children had significant lower levels of 25 hydroxy vitamin D compared to normal children. The elevation of anti macrophage autoantibody levels was seen in 70% of autistic children and there was negative and significant correlation between serum levels of 25 hydroxy vitamin D and anti macrophage autoantibody.

It was suggested that vitamin D deficiency probably had affect on induction of anti macrophage autoantibody in these children (35). Although in the present study the difference between vitamin D levels was not significant between two groups.

Also in some studies, ASD children have significant lower levels of 25 hydroxy vitamin D compared to normal children (36).

Based on the results of Bromely et al. study, vitamin D deficiency during pregnancy is related to increase of risk of ASD in children (37).

The results of Merguid et al. study indicated that ASD Egyptian children had significant lower levels of calcidiol, calciteriol and serum calcium compared to normal controls (21). In the present study serum calcium level was not measured. It is possible that the cause of difference is the combination of vitamin D and calcium deficiencies that it is a cause of presentation of ASD so more researches in this field are necessary.

Fernell et al. measured the serum levels of vitamin D in mothers of children with and without autism in Sweden and Somali. The results showed that Somalia mothers with autistic children had the lowest level of vitamin D but the difference of this level was not significant among Somalia mothers with and without autistic children.

This part of the results was consistent with the present study and perhaps this comparison is not exact in communities with high prevalence of vitamin D deficiency.

Although the results of Molloy et al. study in United States were the same to the present study and the serum levels of vitamin D in children with and without ASD have not any significant difference and the serum levels of vitamin D in all children were lower than 31 ng/dl (39).

According to the controversies in conducted studies about the relation of the serum levels of vitamin D among ASD children with this disorder, more researches in this field are necessary.

According to the role of vitamin D in neuro-developmental disorders such as autism and possibility of adequate and simple supply of it during pregnancy and early childhood, it suggests that future researches will be conduct with more samples of different geographic areas of country to prevent the adverse effects of vitamin D deficiency.

## Conclusion

Based on our findings, vitamin D serum levels in children with and without autism spectrum disorder have no significant difference. Also the level of vitamin D level has no significant relationship with the severity of autism spectrum disorder.

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