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# Evaluation of Serum Vitamin D Level as Risk Factor for Children with Autism Spectrum Disorder

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## Abstract

**Background:** Autism spectrum disorder (ASD) is characterized by deficits in social communication and social interactions, as well as restricted, repetitive patterns of behavior, interests or activities. Vitamin D deficiency has recently been proposed as a possible environmental risk factor for ASD. Vitamin D has a unique role in brain homeostasis, embryogenesis and neurodevelopment & immunological modulation.

**Objective:** To determine the association between serum vitamin D3 level and autism spectrum disorder (ASD) in children.

**Results:** The study was conducted at Institute of Pediatric Neuro-disorder and Autism (IPNA), BSMMU on 68 diagnosed cases of ASD and 34 age matched control patients .The mean age of ASD patients were 47.6 ±23.7 months and that was 46.6 ±21.4 months in control group. Seventy Nine percent patients were in less than 5 years age group in ASD patients and 82% in control group. There were 83% male patients in case group and 82% in control group. Male outnumbered female in both the groups. Male to female ratio was 5.1: 1 and 4.6: 1 in case and control group respectively. The mean serum vitamin D3 level was 23.8 ±7.8 ng/ml in ASD patients and 26.7 ±9.6 ng/ml in the control group and there were no significant difference in two groups ($p>0.05$). In 73.5% ASD cases serum vitamin D3 level was below the normal level (less than 30 ng/dl) and that was 67.6% in control group.

**Conclusion:** The mean serum vitamin D3 level was inadequate in ASD cases thanthe controlgroup.There was no significant difference between two groups. Age, sex, birth place, age of parents, educational status of parents and in economic status, there were no significant difference between case and control.

**Keywords:** Autism Spectrum Disorder; Vitamin D

## Introduction

Autism spectrum disorder (ASD) is characterized by deficits in social communication and social interactions, as well as restricted, repetitive patterns of behavior, interests or activities. Depending on the child's predominant symptomatology, ASD is comprised of autistic disorder and two related but less severe disorders: asperger disorder and pervasive developmental disorder not otherwise specified (PDD-NOS). [1] ASD usually appears during the first three years of life, but some cases appear later in life when social demands increase (regressive subtype). Individuals with ASD have: (1) difficulty in expressing and understanding certain emotions; (2) difficulty in understanding others' mood; (3) impairment in expressive language; (4) abnormal eye contact; (5) prefer minimal changes to routine; and (6) restricted ways of using toys and preference for isolated play, all of which make it difficult for individuals to establish relationships with others, to act in an appropriate way and to live independently. [2] Children with ASD also frequently experience behavioral and medical symptoms.

Over the past few years, the prevalence of ASD has increased dramatically. While previous prevalence studies in early 2000s, identified less than 30-60 in 10,000 individuals, recent US estimates suggest rates of 50-90 in 10,000 children with notable variation according to child age, gender, race and socio economic status. In Bangladesh the overall prevalence rate for ASD is 1.55/1000, and in rural populations it is 0.68/1000 and in Dhaka city 30/1000.[3] The pathophysiological etiologies which precipitate autism symptoms remain elusive and controversial in many cases, but both genetic and environmental factors (and their interactions) have been implicated. [4] Evidence now suggests that the environment may play a significant role in triggering autism, probably not on its own but through a complex interaction with genetic susceptibilities.[5] There is emerging evidence supporting the hypothesis that autism may result from a combination of genetic susceptibility and exposure to environmental toxins at critical moments in brain development.

Vitamin D deficiency has recently been proposed as a possible environmental risk factor for ASD. Vitamin D has a unique role in brain homeostasis, embryogenesis and neurodevelopment, immunological modulation (including the Brain's Immune System), antioxidation, anti-apoptosis, neural differentiation and gene regulation.[6] Children with ASD had significantly lower serum levels of 25-hydroxy Vitamin D (25(OH) D) than healthy children. Therefore, Vitamin D deficiency during pregnancy and early childhood may be an environmental trigger for ASD. [7]

Vitamin D deficiency is a major health problem noticed in many parts of the world. It is not restricted to sunshine-limited regions of the globe. It is still commonly seen in sunshine-rich areas such as Asia-Pacific, Africa and Middle East regions. [8]

Vitamin D regulates a gene responsible for the conversion of tryptophan into serotonin. Serotonin is crucial during fetal brain development. [9] When vitamin D is lacking, tryptophan cannot be converted into serotonin, thus it can produce neurological defects.

The aim of this study was to find an association between Vitamin D level and autism spectrum disorder (ASD) in Children.

## Methodology

This was a comparative cross sectional study. The study was conducted at Department of Paediatric Neurology, Bangabandhu Sheikh Mujib Medical University, Dhaka from July 2017 to June 2018.Total 102 patients were selected consecutively following the inclusion and exclusion criteria. Evaluations of patients were based on history, physical examination and neurodevelopment assessment. For analysis of results patients were divided into two groups: case-68 and control-34.

### Inclusion Criteria

**For case:** Children aged 3 to 15 years who fulfilled the diagnostic criteria of autism spectrum disorders according to DSM-5.

**For control:** Children Age 3 to 15 years who presented to OPD, IPNA, and BSMMU for condition other than autism spectrum disorder.

**Exclusion criteria**

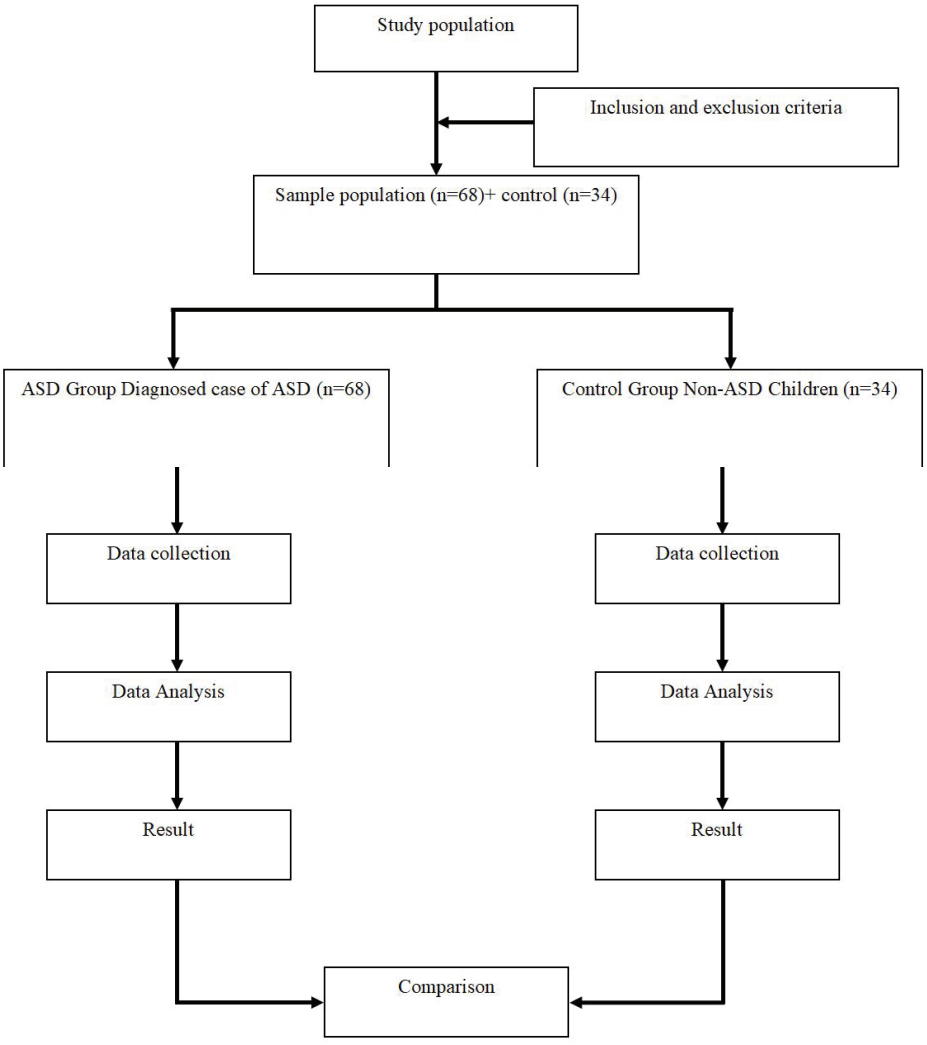
1. Children with ASD who received calcium and or vitamin D therapy in the past 6 months.
2. Children with ASD associated rickets, CKD and other Vitamin D and calcium metabolism disorder.
3. Children with ASD associated with tuberous sclerosis, down syndrome, fragile X syndrome and phenylketonuria were excluded from the study

## Blood collection and serum measurements of Vitamin D

The blood sample (2.5ml venous blood) were collected from case and control group with aseptic way to see serum 25 hydroxy vitamin D levels in the Department of Biochemistry & Molecular Biology, BSMMU.Serum 25(OH) D was measured by chemiluminescencemicroparticle immunoassay in a commercially available kit (CI 4100 ARCHITECT, USA).Vitamin D deficiency which is defined as serum 25(OH) D is lower than 30 ng/ml and optimum levels are between 30 and 80 ng/ml.

Institutional Review Board of BSMMU approved this study. In addition, an informed written consent of participation in the study were signed by the parents or the legal guardians. Data analysis was performed by Statistical Package for Social Science (SPSS), version-17. Then vitamin D level was estimated in ASD children and chi square tests were applied to assess the level of significance. Results were presented as text and tables.

## Flow chart for the steps of study



## Results

To estimate serum vitamin D3 level in children with ASD and to compare the results with children with illness other than ASD who visited OPD of Paediatric Neurology, sixty eight diagnosed cases of ASD were taken as case and thirty four patients who attended with isolated speech delay were enrolled as control group.

| Age group in years | Case | | Control | | p value |
|---|---|---|---|---|---|
| | Frequency | Percentage | Frequency | percentage | |
| <5 | 54 | 79.41 | 28 | 82.35 | 0.75$^{NS}$ |
| 5-10 | 13 | 19.11 | 6 | 17.64 | |
| >10 | 1 | 1.47 | 0 | 0 | |
| Total | 68 | 100 | 34 | 100 | |

Chi-square test was done to measure the level of significance (NS = Non significant)

**Table 1:** Distribution of studied subjects by age (in year)

In both group maximum patients were in less than 5 years age group. They were 79.41% and 82.35% in case and control group respectively. There was no statistical difference in between two groups.

| Sex | Case | | Control | |
|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage |
| Male | 57 | 83.8 | 28 | 82.35 |
| Female | 11 | 19.2 | 6 | 17.65 |
| Total | 68 | 100 | 34 | 100 |
| Male : Female | 5.1 : 1 | | 4.6 : 1 | |

**Table 2:** Distribution of studied subjects by sex

In that two groups male patients outnumbered female. Male patients were 83.8% and 82.35% in case and control group respectively. Male to female ratio was 5.1: 1 in cases and 4.6: 1 in control group.

| Place of birth | Case | | Control | | P value |
|---|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage | |
| Urban | 53 | 77.95 | 23 | 67.65 | 0.134$^{NS}$ |
| Rural | 15 | 22.05 | 11 | 32.35 | |
| Total | 68 | 100 | 34 | 100 | |

Chi-square test was done to measure the level of significance (NS= Non significant)

**Table 3:** Distribution of studied subjects by place of birth

| Age in years | Case | Control | p value |
|---|---|---|---|
| Minimum | 22 | 26 | 0.394 $^{NS}$ |
| Maximum | 46 | 44 | |
| Age range | 24 | 18 | |
| Mean age | 33.7 | 32.82 | |
| Standard deviation | ±5.0 | ±4.66 | |

Unpaired t- test was done to measure the level of significance (NS= Non significant)

**Table 4:** Distribution of studied subjects by age of father at child birth

Regarding place of birth of cases, majority of patients came from urban area. They outnumbered rural category in both case and control groups. Around 78% patients in cases and 68% patients of control group was from urban. But there was no statistically significant different between two groups.

The mean age of father at the time of child birth was 37.7 years in case group and that was 32.82 years in control group respectively. Age range was 24 years in case group and 18 years in control group. Mean difference was not significant in two groups (P>0.05).

| Age in years | Case | Control | p value |
|---|---|---|---|
| Minimum | 17 | 18 | $0.414^{NS}$ |
| Maximum | 36 | 41 | |
| Age range | 19 | 23 | |
| Mean age | 27.38 | 26.55 | |
| Standard deviation | ±4.7 | ±5.07 | |

Unpaired t- test was done to measure the level of significance (NS= Non significant)

**Table 5:** Distribution of studied subjects by age of mother at child birth

The mean age of mother at the time of child birth was 27.38 years in case group and that was 26.55 years in control group respectively. Age range was 19 years in case group and 23 years in control group. Mean difference was not significant in two groups (P>0.05).

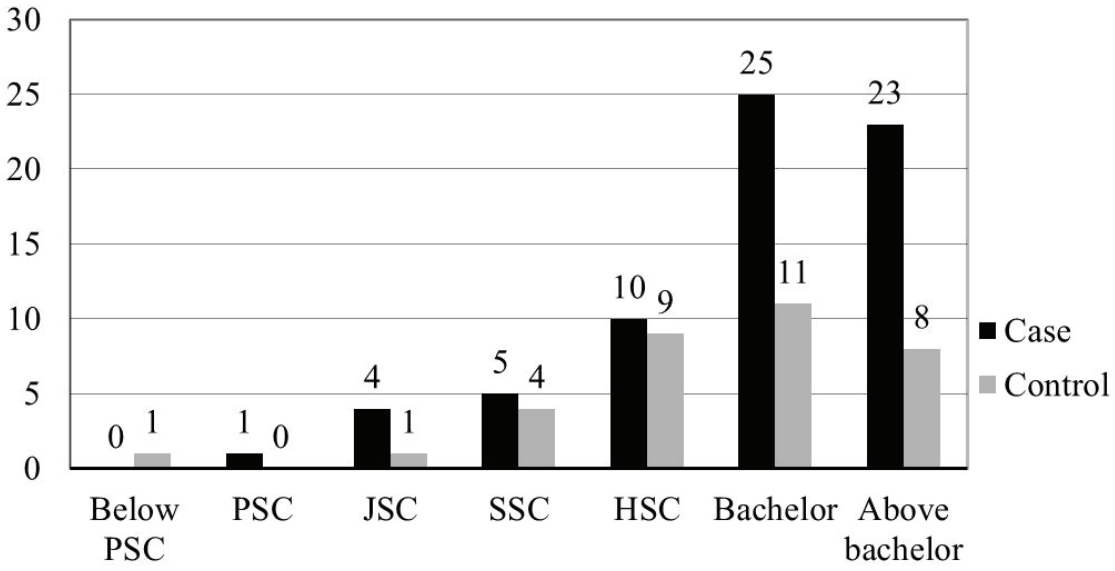



**Figure 1:** Distribution of studied subjects by educational status of father

The educational status of father was plotted as above categories. Majority father of patients was bachelor degree holder they were 25 (36.76%) in case group and 11 (32.35%) in control group. Second majority category was post graduate that was plotted as above bachelor. They were 23 (33.82%) in case group and 8 (23.52%) in control group. Other categories were HSC, JSC, PSC and below PSC.

The educational status of mother was plotted as above categories. Majority mother of patients was bachelor degree holder they were 25 (36.76%) in case group and 16 (47.05%) in control group. Second majority category was post graduate that was plotted as above bachelor. They were 23 (33.88%) in case group and 5 (14.70%) in control group. Other categories were HSC, JSC, PSC and below PSC.

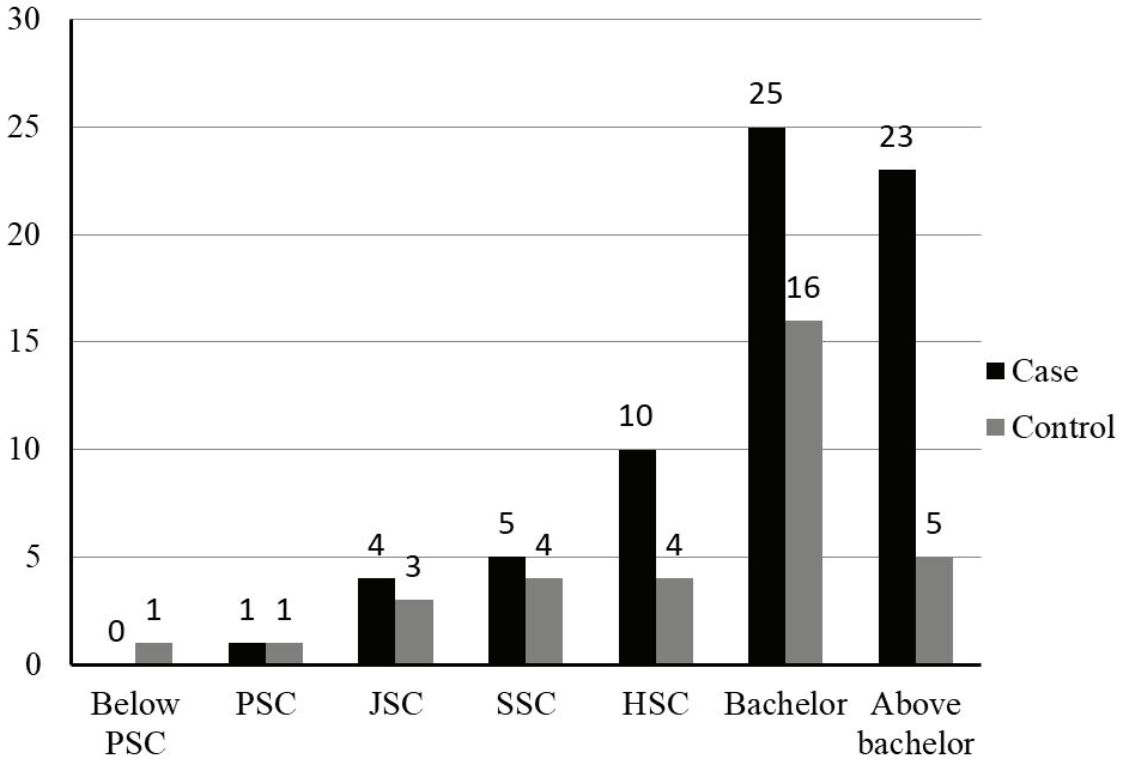



**Figure 2:** Distribution of studied subjects by educational status of mother

| Monthly family income in taka | Case | | Control | | P value |
|---|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage | |
| <10000 | 2 | 2.94 | 0 | 0 | 0.508[NS] |
| 10000-<25000 | 8 | 11.76 | 1 | 2.94 | |
| 25000-50000 | 24 | 35.29 | 14 | 41.17 | |
| >50000 | 34 | 50 | 19 | 55.88 | |
| Total | 68 | 100 | 34 | 100 | |

Chi-square test was done to measure the level of significance (NS= Non significant)

**Table 6:** Distribution of studied subjects by monthly family income

The monthly family income was categorized as less than 10000 taka to more than 50000 taka. Among them 50% patient in case group and 55.88% patients in control group came from affluent family and their monthly& family income was more than 50000 taka. There was no significant difference between the distribution of two groups (P>0.05).

| Environment of residing place | Case | | Control | |
|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage |
| Proper sun-exposed | 42 | 61.76 | 5 | 14.7 |
| Lack of sun exposure | 6 | 8.82 | 29 | 85.29 |
| High rise building | 20 | 29.41 | 0 | 0 |
| Total | 68 | 100 | 34 | 100 |

**Table 7:** Distribution of studied subjects by environment of residing place

Table VII shows the residing places or environment of patients and that was as above distribution. In case group 42 (61%) patients was resided in well sun exposed area and in control group that figure was 5 (14%). In case group 29% patients were resided in high-rise multistoried building where sun light may be insufficient. Lack of proper sunlight exposure was recorded 6 (8.8%) in case group and 85% of control group.

| Serum vitamin D3 level (ng/ml) | Case | | Control | | P value |
|---|---|---|---|---|---|
| | Frequency | Percentage | Frequency | Percentage | |
| 10-30 | 51 | 73.53 | 23 | 67.64 | $0.26^{NS}$ |
| >30 | 17 | 26.47 | 11 | 32.35 | |
| Total | 68 | 100 | 34 | 100 | |

Chi-square test was done to measure the level of significance (NS= Non significant)

**Table 8:** Distribution of studied subjects by serum vitamin $D_3$ level

The serum vitamin D3 was below the normal level in 51 (73.53%) patients in case group and that was 23 (67.64%) patients in control group. There was no statistically significant difference between two groups (P>0.05).

| Serum vitamin D3 level (ng/ml) | Case | Control | p value |
|---|---|---|---|
| Minimum | 9.4 | 12.3 | $0.116^{NS}$ |
| Maximum | 50.3 | 63.7 | |
| Range | 40.9 | 51.4 | |
| Mean level | 23.89 | 26.7 | |
| Standard deviation | ±7.8 | ±9.6 | |

Unpaired t-test was done to measure the level of significance (NS= Non significant)

**Table 9:** Distribution of studied subjects by comparison of serum vitamin $D_3$ level in two groups

The range of serum vitamin D3 level was 40.9 ng /ml in case group and 51.4 ng/ml in control group. Mean serum vitamin D3 level was 23.89 (±7.8) ng/ml in case group and that was 26.7 (±9.6) ng/ml in control group respectively. Two means was compared but there was no statistically significant difference between two groups (P>0.05).

## Discussion

In current study mean age of patients was around 2 years and majority patients were under five years of age and that was 79% in case group and 82% in control group. Male outnumbered female in both groups. Eighty three percent (83%) patients was male in case group and that was 82% in control group respectively. [10] found that prevalence of autism is more in boys than girls (65% vs 55%) that findings is similar with us.

In a similarly designed study the investigators found that the onset of ASD was before 3 years of age. [11] Another group of investigators found 56% patients of ASD was up to 5 years age group and 65% of cases was male and male also outnumbered female. [12]That results were similar with our results though their sample size (n=254) was larger than us. In another study the mean age of diagnosis of ASD was 3.1 (±1.7) years but mean age of our study was near 2 years. [13]It indicated that in our center we did earlier diagnosis of ASD.

Regarding place of birth 78% ASD patients was from urban area and that was 68% in control group. We had a belief that urban people are more prone to develop ASD. Though there was no significant difference between two groups but in Bangladesh another study report showed that the prevalence rate for ASD is more in Dhaka city (30/1000 ) than rural area (0.68/1000).

Educational status of father and mother of two groups were compared in our study. Around 70% father of cases and 55% of that was in control group were graduate and post graduate. In case of mother 70% was obtained graduation and post-graduation degree in case group and that was 61% in control group. From that finding we might say that our cases came from well-educated family or we can say that ASD occurrence is more in highly educated family. But there was no significant difference between two groups. In a similar study authors found educational level of father was in university and above that level was 37% in cases group and that was 39% in control group. They also found the educational level of mother was in university and above level was 28% in case group and that was 21% in control group. They showed that majority percentage of parents was in higher educational level. That finding was similar with our finding but percentage of that category was higher in our parents. Another study showed that the majority 26(44.8%) mother were graduate and above, 17(29.3%) were higher secondary, 10(17.2%) were secondary education level. [14] In another study the investigators found that prevalence of autism was higher in parents with higher educational background.[15]

According to these above study we can make an inference that ASD percentage was higher in well-educated family.

The mean age of father at child birth was 33.7 (±5.0) years in case and that was 32.82 (±4.66) years in control group. Mean age of mother at the child birth in case group was 27.38 (±4.7) years and that was 26.55 (5.07) years in control group. There was no statistically significant difference in two groups. In a cohort study investigator found paternal age at child birth of ASD cases 56% was in 25-34 years group and that was 58% in maternal age at 25-34 years group. [16] In another study showed that the majority (74.2%) of the mothers were aged between 18 and 30 years.[14] Sandin et al. found that advanced maternal age was associated with autism.[17] Those results were supported to our study.

Monthly income of family was one of our variables to judgement socio-economic condition of ASD patients and control. We found that in 50% cases family income was more than 50000 taka and that was 56% in control group. Therefore we might say that our majority ASD cases and also control group was solvent. A similarly designed study found that their majority cases came from middle economic class. [12]

Serum vitamin D3 level estimation and comparison in case and control groups was the main aim of this study. We found low serum vitamin D3 level in 75% ASD patients and 67% in the control group. The mean serum vitamin D3 level was 23.89 ng/ml in ASD patients and that was 26.7 ng/ml in control group. In our study the mean vitamin D3 level is low or insufficient category in both case and control group but statistically there was no significant difference in between two groups. Bener et al. found that serum vitamin D level was 18.39 ng/ml in 254 ASD patients they also found significant difference between control (n=254) group. In a similar study on 70 ASD patients the authors found the mean serum vitamin D3 level was 28.5 ng/ml and that was 40.17 ng/ml in control (n=40) group. [18]In that two different study on ASD cases the investigators found mean serum vitamin D3 level is lower than normal value. That finding was similar with our findings. According to a study of Kocovska et al, ASD children are highly selective in food intake thus they are in risk of development of vitamin D deficiency.[19] Therefore patients of ASD should have assessed their diet screened for vitamin D deficiency. [20] They also considered that autism is a very heterogeneous condition with many etiologies and indicate vitamin D as an important factor among their operating elements.

With other authors, we can draw an inference that in ASD cases serum vitamin D3 level becomes low or there might be an association with ASD and vitamin D3.

## Conclusion

The mean serum vitamin D3 level in ASD cases was lower than the control group but this was not significant. There were no significant difference in age, sex, birth place, age of parents, educational status of parents and economic status between case and control. Therefore, we can conclude that there is no association with serum vitamin D3 level and ASD. Here we can add that Vitamin D3 level is inadequate in children of 3-15 years age range in Bangladesh.

## Limitations of this study

1. The sample size was small

2. Recruitment of the ASD cases and controls were a difficult job, because a good number of parents or caregivers were unwilling to give blood for testing for this study.

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