Reduced Serum Levels of 25-Hydroxy and 1,25-Dihydroxy Vitamin D in Egyptian Children with Autism

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Abstract

Objective: The aim of this study was to investigate the potential role of vitamin D in autism through serum level assessment.

Design: This was a case-controlled cross-sectional study.

Setting: The study was conducted at the Out-patient Clinic for “Children with Special Needs” at the Medical Services Unit of the National Research Centre in Cairo, Egypt.

Subjects: Seventy (70) children with autism diagnosed according to the DSM-IV criteria of the American Psychiatric Association were recruited for this study. The mean age ± standard deviation (SD) of the patients was 5.3 ± 2.8 years. Controls included 42 age-matched randomly selected healthy children of the same socioeconomic status (mean age ± SD, 6.1 ± 1.8 years).

Methods: Circulating levels of both forms of vitamin D (25(OH)D and 1,25(OH)2D) and serum calcium were measured for all subjects. Associations between vitamin D status, birth season, and clinical characteristics of autism were examined.

Results: Children with autism had significantly lower 25(OH)D (p < 0.00001) and 1,25(OH)2D (p < 0.005) as well as lower calcium (p < 0.0001) serum values than the controls. A significant positive correlation was obtained between 25(OH)D and calcium (correlation coefficient r = 0.309, p < 0.01) within the children with autism. No significant difference was found on comparison of birth month and season of birth between children with autism and healthy controls. Furthermore, associations linking parental consanguinity or convulsions with vitamin D could not be established.

Conclusions: Serum values of 25(OH)D in the children with autism of this study could classify them as being “vitamin D inadequate,” which lends support to the hypothesis that autism is a vitamin D deficiency disorder.

Introduction

During the past decade, important advances in the study of vitamin D have been made. In addition to the important role of vitamin D in skeletal development and maintenance, evidence is mounting that it produces a beneficial effect on extraskeletal tissues and that the amounts needed for optimal health are probably higher than previously thought.1 Growing data from numerous studies implicate vitamin D deficiency in various diseases including cancer, cardiovascular disease, hypertension, diabetes, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, mental illness, influenza, autism, asthma, and chronic pain.2–6 Claims that vitamin D may help prevent such a variety of diseases is attributed not only to its single endocrine function as a steroid hormone (the regulation of serum calcium and bone metabolism) but also to its multiple autocrine, and presumably paracrine, functions.9 The enzyme that hydroxylates 25-hydroxy-vitamin D (25(OH)D) to 1,25(OH)2D (activated vitamin D, the steroid hormone) is present in a variety of human tissues other than kidney. Thus, locally produced 1,25(OH)2D exists in most tissues of the body and is under autonomous autocrine control.10 Recent data demonstrating the brain localization of vitamin D 25-hydroxylase and 25-hydroxyvitamin D-1α-hydroxylase enzymes suggest that the central nervous system can locally perform the bioactivation of the vitamin D prohormone.11,12

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and in verbal and nonverbal communication. Recently, the prevalence of autism has increased dramatically, which many agree cannot be attributed completely to improved diagnostic techniques and increased awareness. In 2008, John Cannell stated that it was reasonable to search for neurosteroids that have declined over the same time autism has increased. He also added that, of the neurosteroids involved in brain development, activated vitamin D was unique, the least understood, but, arguably, one of the most profound. McGrath et al. pointed out in 2001 that vitamin D is a potent upregulator of nerve growth factor and that the vitamin D receptor is found in a wide variety of brain tissue very early in embryogenesis. They were the first to conclude that hypovitaminosis D should be examined in more detail as a candidate risk factor for neurodevelopmental disorders. In 2006, Kalueff et al. suggested that vitamin D offers neuroprotection, antiepileptic effects, and immunomodulation, as well as regulation of behaviors. In 2006, Kalueff and Tuohimaa reviewed the brain-enhancing properties of vitamin D in even more detail and concluded that the scientific data stress the importance of perinatal vitamin D adequacy for the mother as well as sufficient vitamin D supplementation for the child for “normal brain functioning.”

Relatively few studies have focused on the vitamin D status of pediatric patients with autism. Therefore, the purpose of this study was to further investigate the potential role of vitamin D in autism by measuring circulating levels of both forms of vitamin D (25(OH)D and 1,25(OH)2D) as well as calcium in children with autistic disorders and comparing them with age-matched normal controls. Associations between vitamin D status, birth season, and clinical characteristics of autism were examined.

Subjects and Methods

Subjects

Seventy (70) children with autism receiving care at the Out-patient Clinic for “Children with Special Needs” at the Medical Services Unit of the National Research Centre in Cairo, Egypt, were recruited. Diagnosis of autism was consistent with DSM-IV (APA, 1994) criteria of the American Psychiatric Association and Autism Diagnostic Interview-Revised (ADI-R). The mean age ± standard deviation (SD) of the patients was 5.3 ± 2.8 years. Parent consanguinity, birth month, as well as presence or absence of convulsions was noted for each child. Controls included 42 age-matched healthy children of the same socioeconomic status (mean age ± SD, 6.1 ± 1.8 years) selected from other clinics at the same facility such as the dental outpatient clinic or vaccination health office. They were then thoroughly screened to exclude any disease or affection that would potentially interfere with this study. Written informed consent from the parent or caregiver was obtained for all participants before the study. The study was approved by the Ethics Review Committee of the National Research Centre.

Analytical measurements

Serum samples were obtained from all patients and controls. Serum calcium was measured on the same day using the automated standard laboratory method. Vitamin D serum samples were stored at −20°C for subsequent analysis. All samples were assessed in duplicates. Both serum 25(OH)D and 1,25(OH)2D were quantified by radioimmunoassay using Immuno Diagnostic Systems kits (IDS, Boldon, UK). The kits use highly specific monoclonal sheep antibodies directed against 25(OH)D and 1,25(OH)2D. The detection limit for 25(OH)D assay was 1.2 ng/mL with intra- and interassay coefficients of variation of 5.1% and 8.2%, respectively. For 1,25(OH)2D assay, the detection limit was 1.4 ng/mL and intra- and interassay coefficients of variation were 9.1% and 9.6%, respectively.

Statistical analysis

Results were expressed as mean ± SD. Statistical differences between groups were analyzed for significance using the unpaired Student’s t test. The Mann–Whitney U test was applied when the distribution was not normal. For the purpose of this study, the birth month of participants was regrouped according to seasons of the year and categorized as winter (December–February), spring (March–May), summer (June–August), and fall (September–November). Analysis of variance was applied to compare between the groups. In addition, χ2 analysis was used to test associations between parent consanguinity, convulsions, and vitamin D within the children with autism. The relationship between parameters was assessed using Spearman’s correlation test. Statistical significance was defined as p < 0.05. All analyses were performed using SPSS statistical software (version 12).

Results

Clinical characteristics of study subjects are presented in Table 1. Age and birth month were statistically similar in both groups (no significant difference, p > 0.05). Serum calcium levels were significantly higher in the controls than in the children with autism (p < 0.0001). Mean 25(OH)D and 1,25(OH)2D levels in healthy children were 40.1 ± 11.8 and 32.8 ± 9.1 ng/mL, respectively. Children with autism had significantly lower 25(OH)D (28.5 ± 16.4 ng/mL, p < 0.00001) and 1,25(OH)2D (27.1 ± 10.7 ng/mL, p < 0.005) serum values than the controls. Correlation analyses confined to the autistic subjects were performed in an attempt to correlate between the various parameters in this study (data not shown). A significant positive correlation was only obtained between 25(OH)D and calcium (Fig. 1) (correlation coefficient r = 0.309, p < 0.01).

No significant difference was found on comparison of birth month and season of birth between children with autism and healthy controls. Figure 2 reveals the frequency of

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children with autism</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>70</td>
<td>42</td>
</tr>
<tr>
<td>Age (years)</td>
<td>5.3 ± 2.8</td>
<td>6.1 ± 1.8</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.9 ± 0.8**</td>
<td>9.5 ± 0.8</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>28.5 ± 16.4***</td>
<td>40.1 ± 11.8</td>
</tr>
<tr>
<td>1,25(OH)2D (ng/mL)</td>
<td>27.1 ± 10.7*</td>
<td>32.8 ± 9.1</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation.

*p < 0.005 versus healthy controls.

**p < 0.0001 versus healthy controls.

***p < 0.00001 versus healthy controls.
birth dates per month of the 70 children with autism. The month of June showed the greatest number of births (18/70; 26.7%) followed by March and April (8/70; 11.4%). Figures 3 and 4 represent box plots of birth season variation in serum 25(OH)D and 1,25(OH)2D, respectively, in autism. No statistical significance was noted between seasons.

Out of 70 children with autism studied, 5 had a history of convulsions and 13 showed parental consanguinity. No associations could be established between the former two variables and other parameters of this study (data not shown).

Discussion

In the current study, we measured the levels of 25(OH)D and 1,25(OH)2D in the serum of children with autism living in Egypt. Our results indicated that children with autism did in fact have significantly lower serum vitamin D levels than their healthy counterparts. In addition, serum calcium was also significantly lower in autism when compared to the controls. Furthermore, a positive correlation was found between calcium and 25(OH)D in the children with autism. Herndon et al. compared the dietary intake of children with autistic-spectrum disorders to that of children with typical development. They noted that large proportions of children did not meet the national recommendations for daily intake of vitamin D and calcium.19 Despite the relatively restricted number of subjects in this study, which cannot be conclusively assumed as a reflection of the total Egyptian pediatric population, our results indicate that the mean 25(OH)D level in the control group was 40.1 (±11.8) ng/mL. Continuous debates in scientific circles are ongoing in an attempt to establish the ideal “normal” 25(OH)D levels. Many studies suggest that a minimum circulating level of 25(OH)D should be more than 30 ng/mL.20–27 Michael Holick quoted vitamin D deficiency as <20 ng/mL and vitamin D inadequacy between 20 and 29 ng/mL.28 Cannell and Hollis recently stated that ideal 25(OH)D levels should be maintained at 40–70 ng/mL year-round.9 Reinhold Vieth reported that “natural levels,” that is, levels found in humans who live or work in the sun, are around 50 ng/mL.29 Thus, from the above data and, because Egypt lies at a latitude of 30°N and is sunny year-round, the children with autism in this study with mean 25(OH)D levels of 28.5 (±16.4) ng/mL should be classified as “vitamin D inadequate.” In addition, due to the medical
problems associated with autism, these children may not be spending enough time outdoors.

In a recent study using experimental animals, a group of Australian scientists found that severe maternal vitamin D deficiency in rats produces offspring with aberrant apoptosis and abnormal cell proliferation, reduced expression of a number of genes involved in neuronal structure, hyperlocomotion, and alterations in both learning and memory. Furthermore, a French group found that developmental vitamin D deficiency disrupts 36 proteins involved in mammalian brain development. Severe gestational vitamin D deficiency in rats produces pups with increased brain size and enlarged ventricles, anatomical abnormalities similar to those found in autism. Both the brain and the blood of autistic individuals show evidence of ongoing chronic inflammation and oxidative stress. Subjects with autism show increases in inflammatory cytokines, which have consistently been associated with cognitive impairment. These inflammatory mediators show similarity to the immune processes regulated by vitamin D.

Seizures are common in autism, and activated vitamin D significantly increases the seizure threshold, making the brain tissue less likely to seize. Maintaining vitamin D sufficiency in utero and during early life, to satisfy the vitamin D receptor transcriptional activity in the brain, may be important for brain development as well as for maintenance of mental function later in life. Only 5 children in this study had a history of convulsions. Although their mean serum vitamin D levels were comparatively lower than the mean of those without convulsions, no statistical significance could be established. A much greater sample size is required to further investigate this point.

The frequency of birth months in this study was determined. The month of June showed the most births: 18 of the 70 children with autism (25.7%), followed by March and April with 8 births each (11.4%). Children were further regrouped according to season of birth. Thirty-three percent (33%) of births were in the summer. This is to be expected since the month of June was grouped in this study with summer. These findings are contradictory to other studies on season of birth and autism. Stevens et al. reviewed the literature and noted that at least seven studies found excessive autism births in the winter, especially March when vitamin D levels are at their lowest. In the present study, vitamin D levels were lowest during the summer birth season, although no statistical significance was found.

Conclusions

In conclusion, our case-controlled cross-sectional study reflects the fundamental importance of vitamin D in autism. This study clearly shows that circulating vitamin D levels (both 25(OH)D and 1,25(OH)2D) are significantly lower in children with autism than in healthy controls. Furthermore, serum values of 25(OH)D in these patients could classify them as being “vitamin D inadequate.” These findings suggest that vitamin D insufficiency may play a role in the etiology of autism. Supplementation with vitamin D3 may be extremely important in treating children with autism since their diets tend to be very restricted and they may not be consuming enough vitamin D. However, the small sample size in this study can be cited as one of its limitations. Further extensive research is warranted to explore the potential clinical implications of maintaining high circulating vitamin D levels in infants and children and to evaluate the full therapeutic and preventive potential of vitamin D and its analogues in autism. In addition, association between severity of autism symptoms and vitamin D levels should also be addressed. Vitamin D may be the new frontier for autism prevention and treatment.

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References


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