Impact of Vitamin D among children with autistic spectrum disorder; The value of Childhood Autism Rating Scale (CARS)

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ABSTRACT:

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by a wide spectrum of deficits in social interaction, communication, and behavior. There are many hypotheses regarding causation of ASD but the exact etiology of ASD remain unknown. The presence of vitamin D receptors in the hippocampus, hypothalamus, thalamus, cortex, and substantia nigra may indicate a possible role for vitamin D in the pathophysiology of ASD.

This study aimed to assess the correlation between ASD and vitamin D deficiency and to assess the possible correlation between this vitamin and each item in Childhood Autism Rating Scale (CARS) as negative or positive correlation.

The study included 14 children with ASD (4 female & 10 male) from Basra city in period from May 2020-October in the same year, their age ranged from (6-12 years). They were classified into mild to moderate and sever using the Childhood Autism Rating Scale (CARS) Arabic version. They were free of any congenital anomalies, no history of head injury, no family history of psychiatric disorders.

The comparative data reveals that vitamin D level had significant negative correlation with 5 items of CARS (relation to people, body use, adaption to change, visual response and verbal response), P value < 0.05.

Key words: Autism Spectrum Disorder, CARS, Vitamin D.
INTRODUCTION:
Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is comprised of different subgroups, including Autistic Disorder, Asperger Syndrome and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS)\(^{(1)}\). Although the specific etiologies of ASD remain unknown, many hypotheses regarding causation of ASD abound, there is a significant genetic component to ASD, yet no single gene is responsible for autism spectrum disorder \(^{(2,3)}\). Additionally, many environmental factors play a role like parental age, medication used during pregnancy, maternal smoking and alcohol consumption and vaccination \(^{(4)}\).

Several studies have found a correlation between the presence of circulating maternal autoantibodies and neuronal dysfunction in the neonate. Specifically, maternal anti-brain autoantibodies, which may access the fetal compartment during gestation, have been identified as one risk factor for developing ASD. The specific process of anti-brain autoantibody generation is unclear and the detailed pathogenic mechanisms are currently unknown \(^{(5)}\), evidence of abnormalities in inflammatory markers, autoantibodies has been reported in brains of ASD patients. These findings suggest that ASD is a systemic condition with the likelihood of being a disease of inflammation, autoimmunity, and/or oxidative stress \(^{(6,7)}\).

In humans, vitamin D is obtained mainly from skin exposure to ultraviolet ray (UVB) radiation as its amount in the usual daily diet is limited\(^{(8)}\), it plays an essential role in modulating immune function, cell proliferation and apoptosis, brain development and function, and has also been found to have neuroprotective properties\(^{(9,10)}\).

The receptors for Vitamin D (VDR) and the enzymes involved in its metabolism have been identified in several regions of the brain including neurons and glial cells\(^{(11)}\). VDR has been shown to be present early in development, increase in number through development and, for sure, present in the adult brain which points to the role for vitamin D in the developing of adult brain \(^{(12)}\). Subsequently, vitamin D deficiency has been implicated in pathophysiology of ASD in several ways. It has been hypothesized that ASD is a combination of both organ specific physiologic and systematic abnormalities such as de
novo gene mutations, oxidative stress, impaired detoxification system, inflammation, immune dysregulation, abnormal neurotrophic factor and neurotransmitter levels, and seizures, at least in a subset of individuals with ASD \(^{(13)}\). And there is a mounting evidence suggests that low vitamin D status is involved in the etiology of these mentioned abnormalities\(^{(14)}\).

The Childhood Autism Rating Scale (CARS) is a 15-item observation-based rating scale designed to accurately differentiate children with autism from those with developmental delays without features of autism\(^{(15)}\). It is intended for use by highly trained raters in the context of a wider multi-method approach that includes behavioral observations, interview of primary caregivers, assessment of intellectual functioning, and detailed developmental and family history\(^{(16)}\). It is based on rating the frequency, intensity, duration and atypicality of the specified behavior, while considering the chronological age of the child. Each of the 15 items is rated on a seven-point scale (1, 1.5, 2, 2.5, 3, 3.5, 4) ranging from scale 1 “within normal limits for that age,” to scale 4 “severely abnormal for that age”\(^{(17)}\). A total score is determined by summating the all the 15 items ratings and range from 15-60. Fifteen indicates a child within normal limits for all items while sixty indicates a child with severe abnormality for all items of CARS \(^{(17,18)}\).

In 1980 Schopler and colleagues released the first Edition of CARS, then in 2010 they released CARS 2 \(^{(19)}\) which included a Standard Form (CARS2-ST, previously named the CARS), a High Functioning Version (CARS2-HF) and a Questionnaire for Parents or Caregivers (CARS2- QPC)\(^{(19)}\).

**Aim:**
This study aimed to assess the correlation between ASD and vitamin D deficiency and to assess the connection between this vitamin and each item in Childhood Autism Rating Scale (CARS).

**Material and methods**
Fourteen child (4 females and 10 males) who had been diagnosed with ASD were included in this study the diagnosis confirmed by DSM-5 criteria. Their age ranges from 6 to 12 years. They were free from any congenital anomalies. All were referred from psychiatrists,
pediatricians, and neurologists to a specialized neurophysiology clinic in (Basrah) city where the study was done from the period from May 2020 to October 2020.

The parents (at least one of them) for all participants had signed the study consent that contains a summary of the procedures that the child will pass through including all the questions that will be asked to the participant.

Full history about each participant was taken, concentrating on the 15-item of CARS using preset forma (Fig.1). Additionally, electroencephalogram (EEG) test was done to all.

At the start, most of the participants were uncooperative, but with patience and reassurance good results were achieved. Blood samples were collected while the patient is sleeping during EEG test.

EEG test
The test take about 20-30 min using computerized EEG system 2014 device was used for this purpose. it consists of amplifier connected to the PC unit. It is obtained via CADWELL electronics, 24 channels system, using 10-20 double banana montage. Also, a network of cub electrode cables, neoprene elastic cap with rubber chin strap and adhesive conductive paste (Ten20) were used.

Firstly the family or care giver consent is obtained before starting the test, the participant and his family were reassured that the test is painless he child to be tested was rested and lied comfortably at 45° half sitting position on the couch. The room temperature was kept around 25 C°. Still, most of children were uncooperative and became anxious for touching and refuse wearing the headcap. That is why, ChloraHydrate syrup (30-50 mg/kg of body weight) was used and the test has been done only when the participant go sleep. This was important to ensure accuracy even in painless procedures\(^{(20)}\).

After the EEG and while the participant still sleeping a blood sample was taken to measure vitamin D level.
The childhood autism rating scale (CARS) is a 15-item behavioral rating scale developed to identify children with ASD and categorize these behaviors from mild to moderate to severe. The total CARS score may range from a low of 15 (obtained when the child’s behavior is rated as falling within normal limits on all 15 scales) to high of 60 (obtained when child’s behavior is rated as severely abnormal on all 15 scales). The score represents placement on a continuum: the lower the score, the fewer autistic behaviors the child exhibits; the higher the score, the more autistic behavior the child exhibits.

The scores were as follows:
- Relating to people/
- Emotional response/
- Imitation/
- Body use/
- Object use/
- Adaptation to change/
- Listening response/
- Taste, smell, touch/
- Visual response/
- Fear or nervous/
- Verbal communication/
- Activity level/
- Nonverbal communication/
- Level & consistency of intellectual response/
- General impression/
- Total score/

**Figure 1:** The history taking form
Statistical analysis was performed in this study using Statistical Package for Social Science - Version 19 (SPSS).

Independent t-test was used to estimate differences between two groups in continuous variables. Also one-way ANOVA was used to evaluate differences of means among multiple groups. Correlation between measured parameters and other clinical parameters were analyzed by Pearson's correlation analysis.

Results:

The patients ages range from 6-12 years in mean of 7.4±1.4 , BMI range from (10.65-24.20) in mean of 18.15, CARS test range from (28.50-41.50) in mean of 34.46.

Table 1: Patient age, gender, EEG, BMI & CARS

<table>
<thead>
<tr>
<th>Patient's characteristics</th>
<th>NO.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-7</td>
<td>9</td>
<td>64.3</td>
</tr>
<tr>
<td>8-11</td>
<td>5</td>
<td>35.7</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>71.4</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>EEG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>57.1</td>
</tr>
<tr>
<td>Abnormal</td>
<td>6</td>
<td>42.9</td>
</tr>
<tr>
<td>Mean age± SD</td>
<td>7.4±1.4</td>
<td></td>
</tr>
<tr>
<td>Mean BMI±SD</td>
<td>18.2±3.2</td>
<td></td>
</tr>
<tr>
<td>Mean CARS±SD</td>
<td>34.5±4</td>
<td></td>
</tr>
</tbody>
</table>

In try to find a connection between the CARS test values in different patients to EEG finding, so summarize the results in histogram as in figure below, that there are no significant difference between normal and abnormal EEG regarding the CARS values.
Figure 2: Comparison of mean of CARS test between normal and abnormal EEG
The correlation between vit D level and CARS test is moderate correlation (0.58 and P value < 0.05) then correlate each item of the CARS test and vit D.

Table 2: the correlation between vit D and CARS test items

<table>
<thead>
<tr>
<th>Item</th>
<th>Coefficient of correlation (r)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARS</td>
<td>-.589-*</td>
<td>.027</td>
</tr>
<tr>
<td>Relation to people</td>
<td>-.719-**</td>
<td>.004</td>
</tr>
<tr>
<td>Emotion</td>
<td>.251</td>
<td>.387</td>
</tr>
<tr>
<td>Imitation</td>
<td>-.189-</td>
<td>.517</td>
</tr>
<tr>
<td>Body use</td>
<td>-.799-**</td>
<td>.001</td>
</tr>
<tr>
<td>Object use</td>
<td>-.438-</td>
<td>.117</td>
</tr>
<tr>
<td>Adaption to change</td>
<td>.079</td>
<td>.790</td>
</tr>
<tr>
<td>Listen response</td>
<td>-.209-</td>
<td>.472</td>
</tr>
<tr>
<td>Taste, smell &amp; touch response</td>
<td>-.041-</td>
<td>.889</td>
</tr>
<tr>
<td>Visual response</td>
<td>-.617-*</td>
<td>.019</td>
</tr>
<tr>
<td>Fear</td>
<td>-.464-</td>
<td>.095</td>
</tr>
<tr>
<td>Verbal</td>
<td>-.695-**</td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td>P value</td>
<td>Coefficient of correlation (r)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Activity level</td>
<td>.006</td>
<td>-.194</td>
</tr>
<tr>
<td>P value</td>
<td>.505</td>
<td></td>
</tr>
<tr>
<td>Nonverbal communication</td>
<td>.330</td>
<td>-.282</td>
</tr>
<tr>
<td>P value</td>
<td>.344</td>
<td></td>
</tr>
<tr>
<td>Intellectual response</td>
<td>.304</td>
<td>-.296</td>
</tr>
<tr>
<td>General impression</td>
<td>.228</td>
<td>-.344</td>
</tr>
</tbody>
</table>

5 items of CARS test had significant negative correlation to vit D level, these are relation to people, body use, adaption to change, visual response and verbal response.

![Figure (3): correlation of vit D to relation to people in CARS test in autism](image-url)
Figure (4): the correlation of vit D to body use in CARS test in autism spectrum disorder

Figure (5): the correlation of vit D to visual response of CARS test of autism spectrum disorder
Figure (6):the correlation of vit D to verbal communication of CARS test of autism spectrum disorder

**Discussion:**

Autism is clearly a multi-system disorder that impacts the brain, the immune system, the gastrointestinal tract, and other organ systems and Vit D which is one of the vital vitamin that work in multi directions.

Basically it comes from exposure to sun light or taken orally both had the same active product to do the work.

The presence of VDR in the hippocampus, hypothalamus, thalamus, cortex, and substantia nigra prompted many studies on the possible determinant role of vitamin D in different neurological condition \(^{(21)}\). It has been evidenced that calcitriol is a fundamental actor in the neuronal differentiation and the neural maturation \(^{(22)}\).
Vitamin D yields both genomic and non-genomic actions; the Vitamin D Receptors (VDR) mediates one of the representatives of the steroid hormone superfamily, which are evident in more than 30 human tissues, therefore regulating 3% of the human genome (approximately 700 genes) (23). Nuclear VDRs are found in most cells, and support the role for the extraskeletal benefits of vitamin (24) Vitamin D deficiency in early infancy affects differentiation of nervous system, connectivity of axons and development of structure and function of CNS (25), the prevalence of autism has been rising which attributed to multiple factors such as genetic predisposing namely genetic polymorphisms of cytochrome P450 enzymes specifically CYP27B1 (found in mitochondria of cells of our body) which have been involved in pathogenesis of autism and considered as essential for proper vitamin D metabolism.

Serotonin and vitamin D have been suspected to be involved in pathophysiology of autism, but no known mechanism has been reported. Calcitriol (vitamin D hormone) can activate the transcription of the serotonin-synthesizing gene tryptophan hydroxylase 2 (TPH2) in the CNS at a vitamin D response element. So many studies suggested relation between low level of serotonin and Vit D with the repetitive behavior and change in mood.

There are other explanations to the behavior in ASD patients in relation to Vit D role in our bodies one of them is inflammation, in ASD patients the difference between systemic inflammation and neuroinflammation is reflected by the fact that some cytokines are differentially expressed in the brain versus systemically. Increased TGF-ß (transforming growth factor ß) levels are measured in the cerebellum of ASD patients, in contrast to decreased levels in the cerebrospinal fluid or the periphery (26). Upon cell death, cells often secrete TGF-ß to reduce local inflammation. Neurons that showed degeneration were high in TGF-ß, suggesting the increased TGF-ß levels found in the brain of ASD patients are targeted at controlling neuroinflammation. Increased microglial activation, combined with increased pro-inflammatory cytokines results in neuroinflammation (27). This is observed in a large fraction of all ASD cases and could lead to impaired connectivity in the CNS, resulting in the pathophysiology observed in ASD patients. Moreover, oxidative stress is increased in ASD, those children are considered more vulnerable to oxidative stress because of their imbalance in intracellular and extracellular glutathione levels and decreased glutathione.
reserve capacity. Several studies have suggested that the redox imbalance and oxidative stress are integral parts of ASD pathophysiology. As such, early assessment and treatment of antioxidant status may result in a better prognosis as it could decrease the oxidative stress in the brain before it can induce more irreversible brain damage (28).

ASD are associated with aberrant connectivity, which may combine over and under-connectivity, structural and functional data revealed a connectivity disturbance, affecting frontal, fronto-temporal, fronto-limbic, fronto-parietal, and inter-hemispheric connections (29,30).

These areas are related to mental and cognitive function of the brain in motor and sensory function of cerebral hemisphere that appear clear in abnormal relating to people, emotion response, body movements and visual response and others, in these areas of brain the vit D receptors is available as mention before and effective in connectivity among different brain parts so the correlation of certain items in CARS test with the level of vitamin D is expected which proved in this study by negative moderate to strong correlation between serum vit D level and CARS items which show by low vit level increase CARS test or its items values.
REFERENCES


