

Prevalence of Autism Spectrum Disorders in Vitamin D Deficient or Insufficient Rickets

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Short Title: Autism in Vitamin D Deficient / Insufficient Rickets

Abstract: *Background: Autism is increasing, as is vitamin D deficiency; autism epidemic came upon the world at the same time the major health authorities advised fair skin people to avoid the sun, so a link to explore between the 2 conditions appeared tempting to many investigators. Objective: Investigation of the prevalence of Autism Spectrum Disorders (ASDs) in an Egyptian sample of vitamin D deficient/insufficient rachitic infants and children compared to age and sex matched healthy controls. Methodology: Thirty five vitamin D deficient/insufficient rachitic Egyptian infants and children (group I) and 35 clinically healthy age and sex matched controls were enrolled (group II). Rickets biochemical markers, 25 hydroxy vitamin D, and Vineland Adaptive Behavioral Scales were assessed for all studied infants and children. DSM IV TR criteria were used for diagnosis of ASDs that were rated using Childhood Autism Rating Scale (CARS). Results: Mild to moderate autism was recorded in 25.71% of group I compared to none of controls and their CARS total score was significantly negatively correlated with serum level of 25 hydroxy vitamin D. Conclusion: Screening for ASDs in cases of Vitamin D deficient/insufficient rickets, as proved to be a high risk group for developing such disorders, is worthy.*

Keywords: Autism spectrum disorders (ASDs), vitamin D, Childhood Autism Rating Scale (CARS), Vineland Adaptive Behavior Scales (VABS), rickets

1. Introduction

There is an agreement among all professionals that autism is one of the most puzzling diseases. It is a complex neuro-developmental disorder, the prevalence of which has surged in the last 2 decades [1]. It is usually diagnosed before the age of three years with striking 4:1 male to female ratio [2]. It is characterized by defiance in social reciprocity and in language skills that are associated with repetition behavior and restricted interests [3]. In addition to behavioral impairment autistic persons have a high prevalence of gastrointestinal diseases [4], autoimmune diseases [5], and mental retardation [6].

On the other hand, Vitamin D deficiency has been claimed to become an epidemic again in children [7]. It is highly prevalent among children and adolescents worldwide. It could result from inadequate vitamin D acquisition through either poor dietary intake or limited sunlight exposure that leads to depletion of vitamin D stores [8]. The problem of vitamin D deficiency during childhood is of public health relevance, given the growing evidences that vitamin D deficiency may play a key role in the pathophysiology of many chronic diseases; autism included [9].

The apparent increase in the prevalence of autism over the last 20 years corresponds with the increasing medical advice to avoid the sun, an advice that has probably lowered vitamin D level. Autism is more common in areas of limited sun exposure such as urban areas and areas of high air pollution. It is also more common in dark skinned persons and in infants exposed to severe maternal vitamin

D deficiency in utero. Such epidemiological distribution of autism would theoretically greatly lower activated vitamin D "calcitriol" levels especially in developing brains. Interestingly, children with vitamin D deficiency rickets have several autistic markers that apparently disappear with high dose vitamin D treatment [10].

2. Problem Definition

Cannell (2008) [10] hypothesized that simple Gaussian distributions of the enzyme that activates neural calcitriol combined with widespread gestational and or early childhood vitamin D deficiency may explain both the genetics and epidemiology of autism. If so, he claimed, much of the disease is iatrogenic brought on by medical advice to avoid the sun. Accordingly he recommended different studies to test his theory.

3. Study Objectives

The current study was designed to investigate the prevalence of ASDs in an Egyptian sample of vitamin D deficient/insufficient rachitic infants and children compared to age and sex matched healthy controls and to correlate the level of 25 hydroxy vitamin D with the severity of autistic manifestations if any, using Childhood Autism Rating Scale (CARS). To authors' best knowledge, this issue has not been explored in our country before.

4. Study Design and Research Methodology

The current cross sectional study was carried out in accordance to the code of ethics of the **World Medical Association (Declaration of Helsinki, 1989)** [11] for experiments involving humans. Written informed consent of legal caregivers of enrolled infants and children was taken and the study protocol was approved by Ain Shams Faculty of Medicine Ethical Committee.

Participants:

Seventy Egyptian infants and children aged between 1-3 years of both sexes, whose caregivers agreed to participate in the study after explaining its objective to them, were enrolled. They were consecutively recruited from infants and children attending the Outpatient Clinic, Children's Hospital, Ain Shams University. They were classified into the following groups:

Group I (Vitamin D deficient/insufficient rachitic infants & children):

It included 35 untreated clinically and radiologically diagnosed active rachitic infants and children. They were 17 males (48.60%) and 18 females (51.40%) with a male to female ratio of 0.94:1. Their mean age was 2.13 ± 0.67 years. Those who had any concomitant acute or chronic physical illness or handicap, those with radiological evidence of healing or healed rickets, those who have been proven to have adequate level of 25 hydroxy vitamin D, and those who have taken any form of therapeutic doses of vitamin D prior to the study were excluded.

Group II (controls):

It included 35 healthy infants and children; consecutively recruited from infants and children attending the Outpatient Clinic, Children's Hospital, Ain Shams University for growth monitoring or regular check-up. Their mean age was 2.02 ± 0.75 years; 18 were males (51.40%) and 17 were females (48.60%).

Procedure:

All enrolled infants and children were subjected to the following:

1-Thorough clinical history taking laying stress on dietetic history: type of feeding (breast fed with no vitamin D supplementation, breast fed with vitamin D supplementation, mixed bottle and breast feeding, or bottle only, age of weaning, type of food introduced with weaning), history of vitamin supplementation (type of vitamin, time of supplementation, dose, duration of supplementation) and history of calcium and vitamin D supplementation to the mother during pregnancy, history of sunlight exposure (UVR) and its duration, dressing habits, and detailed developmental history [12].

2-Full Clinical Examination with special emphasis on rachitic manifestations (bone tenderness, dental eruption time and problems, muscle weakness (rickety myopathy),

increased tendency for fractures (easily broken bones), especially greenstick fractures, skeletal deformities (bowed legs; genu varum, knock-knees; genu valgum) or "windswept knees", cranial deformity (such as skull bossing or delayed fontanel closure), pelvic deformity, spinal deformity (such as kypho-scoliosis or lumbar lordosis), costochondral swelling "rachitic rosary", double malleoli sign (Marfan's sign) due to metaphyseal hyperplasia, and widening of wrist joint due to metaphyseal cartilage hyperplasia [13]. All body systems examination was also carried out to exclude any concomitant acute or chronic physical illness or handicap.

3. DSM IV TR (2000) diagnostic criteria for ASDs were used to settle the diagnosis of these disorders [14, 15].

4. Psychometric Assessment:

➤ **An Arabic Validated Version of CARS (Childhood Autism Rating Scale)** was used for both identification and rating of autism [16]. This test can be used to determine the severity of autistic symptomatology and can thus be useful in its periodic monitoring. CARS test consists of 15 items, each rated on a 4-point scale (may be extended to 7 points by insertion of intermediate points). The child may be rated between two descriptions by using rating of 1.5, 2.5 or 3.5. The items of CARS include relation to people, imitation, emotional response, body use, object use, adaptation to change visual response, listening response, taste, smell and touch response and use, fear or nervousness, verbal communication, non verbal communication, activity level, level and consistency of intellectual response, and general impressions. The total score of the test can range from 15 to 60 points according to severity of autism. The score can be categorized into: non-autistic (15-29 points) as grade 0, mild to moderately autistic (30-36 points) as grade 1 and severely autistic (37-60 points) as grade 2 [17, 18].

➤ **Vineland Adaptive Behavioral Scales (VABS);** an Arabic validated version of which was used in assessment of enrolled infants and children as it forms an aid in diagnosing and classifying intellectual disability and other disorders such as autism, Asperger syndrome, and developmental delays [19]. It is a diagnostic tool that helps measuring the capabilities of both children and adults in dealing with everyday life. Its content and scales are organized within a 3 domains structure: communication, daily living, and socialization. It offers also, a motor skills' domain and an optional maladaptive behavior index. VABS composite-standard scores have a mean of 100 and a SD of 15 and its sub domains' have means of 15 with SD of 3 [20, 21].

5. Biological Markers of Rickets:

Blood Samples: A venous blood sample was drained from enrolled infants and children under complete aseptic conditions and was allowed to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000 g, serum was removed and assayed to measure:

➤ 25-hydroxy vitamin D concentrations using ELISA technique utilizing Glory Science CO, Ltd 25-OH-VD ELISA Kit. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of 25-OH-VD in the sample, the used kit included a set of calibration standards. The calibration standards were assayed at the same time as the samples and allowed the operator to produce a standard curve of optical density versus 25-OH-VD concentration, the concentration of which was then determined by comparing the optical density of the studied samples to the standard curve. The half life of 25 OH vitamin D is 2-3 weeks that is much longer than that of its metabolite 1,25 (OH)₂ vitamin D which is 4 hours. Accordingly 25OH vitamin D is considered a much better indicator of vitamin D stores and thus it has been chosen for our research. 25-OH-VD levels ≤27.5nmol/L were considered deficient, 28 to 50nmol/L were considered insufficient, and >50nmol/L were considered sufficient [22, 23].

➤ Total serum calcium (normal levels range from 9-10.5 mg/dl), total serum phosphorus (normal levels range from 4.5-5.5 mg/dl), and total serum alkaline phosphatase (normal levels range from 35 - 462 IU/L) [24].

6. Radiological Diagnosis

Plain X-ray of ends of long bones were done for enrolled rachitic cases to insure that all enrolled rachitic infants and children had radiological signs of active rickets (generalized osteopenia, metaphyseal widening, cupping, and fraying due to exaggerated normal concavity and irregular calcification [25].

Data Analysis:

Analysis of the obtained data was done by IBM computer using SPSS (statistical program for social science version 16) [26] as follows: description of quantitative variables as means, SDs, and ranges and description of qualitative variables as numbers and percentages. Chi-square test was used to compare qualitative variables between studied groups. Unpaired t-test was used to compare quantitative variables. Spearman Correlation coefficient "r" test was used to rank quantitative variables versus each other positively or inversely. At the study sample, the calculated study power was 0.80. Results were considered statistically insignificant at $p > 0.05$, significant at $p < 0.05$, and highly significant at $p < 0.01$.

5. Results

Analysis of the obtained data showed statistically non significant differences between studied rachitic cases and controls as regards age at time of being enrolled in the study, age at onset of weaning, and frequency distribution of sex, different types of milk feeding (breast fed, formula fed, mixed), style of weaning (adequate or inadequate vitamin D supplementation), and adequacy of maternal intake of vitamin D and calcium ($p > 0.05$ of all). On the other hand, the habit of completely covering babies with

subsequent poor sunlight exposure was significantly more encountered among rachitic cases (19 cases = 54.29%) compared to controls (5 controls = 14.28%); $p < 0.01$.

Among studied cases, Marfan's sign was the most frequently recorded rachitic manifestation (25 cases = 71.43%), followed by leg bowing (17 cases = 48.57%), delayed dentition (14 cases = 40%), and delayed motor milestones (8 cases = 22.86%). Recurrent respiratory tract infections was the commonest complication reported in enrolled cases (13 cases = 37.14%) followed by recurrent diarrhea (8 cases = 22.86%).

Using DSM IV TR diagnostic criteria, ASDs were diagnosed in 9 rachitic cases (25.71%) who were rated as having mild to moderate autism according to total CARS score compared to none of controls; $p < 0.01$ (Fig 1). Meanwhile, the mean total CARS score of studied rachitic cases (group I mean = 32.79 ± 2.12) was significantly higher than that of controls (group II mean = 20.77 ± 2.77); $p < 0.001$, Table (1). On the other hand, the total VABS score was significantly lower in studied rachitic cases (group I mean = 81.2 ± 15.3) compared to controls (group II mean = 95.1 ± 14.2); $p < 0.05$, Table (1). Also, rachitic autistic cases had significantly lower mean communication VABS scale score compared to controls ($p < 0.001$) and lower mean social VABS scale score compared to both controls and rachitic non autistic cases ($p < 0.01$) for both; Table (2).

Studied rachitic cases had statistically significant reduction of mean values of serum phosphorus and calcium and significant rise of serum alkaline phosphatase compared to controls ($p < 0.05$, < 0.001 , and < 0.001 respectively). Meanwhile, they had significantly lower mean value of 25 (OH) vitamin D compared to controls; ($p < 0.001$); Table (1). As regards vitamin D status distribution, 25 (OH) vitamin D was found to be insufficient in 45.71% and deficient in 54.29% of studied rachitic cases compared to none of controls; $p < 0.01$ for both; Fig (2). On the other hand, deficient and insufficient 25(OH) vitamin D level were recorded in 55.56%, 44.44% of autistic rachitic cases respectively compared to 34.62%, 65.38% of non autistic ones respectively; Fig (3).

In studied rachitic cases, 25 (OH) vitamin D was significantly negatively correlated with age, serum alkaline phosphatase, and total CARS score meaning that the lower the 25 (OH) vitamin D, the older the child, the higher the alkaline phosphatase, and the higher the total CARS score i.e. the severer the autistic manifestations; Table (3) & Fig (4,5) ($p < 0.05$ for all). On the other hand, studied cases showed statistically insignificant association between both 25(OH) vitamin D level and total CARS scores with adequacy of maternal intake of vitamin D and calcium; $p > 0.05$ for both. Also, their total CARS score showed statistically insignificant association with the type of infant milk feeding and style of weaning; $p > 0.05$ for both.

Table 1: Statistical comparison between enrolled groups as regards the mean values of the studied psychometric and biological variables:

Group Variables	Group I Rachitic cases (No = 35)		Group II Controls (No =35)		P	Sig
	Mean	±SD	Mean	±SD		
Total CARS score	32.79	±2.12	20.77	±2.77	<0.001	HS
Total VABS score	81.2	±15.3	95.1	±14.2	<0.05	S
Serum calcium in mg/dl	7.91	±0.76	9.55	±0.66	<0.001	HS
Serum phosphorous in mg/dl	2.81	±0.54	4.67	±2.53	<0.05	S
Serum alkaline phosphatase in IU/L	708.17	±187.54	73.23	±15.81	<0.001	HS
25 (OH) vitamin D in nmol/L	32.60	±11.18	75.20	±18.54	<0.001	HS

t = unpaired t-test was used for statistical comparison
 CARS = Childhood Autism Rating Scale, VABS = Vineland Adaptive Behavior Scales
 P<0.05 = statistically significant, p<0.01 = statistically highly significant

Table 2: Statistical comparison between enrolled groups and subgroups as regards the mean values of VABS domains scores:

Group Variables	Rachitic Autistic Cases (No = 9)		Rachitic Non Autistic Cases (No = 26)		Controls (No =35)	
	Mean	±SD	Mean	±SD	Mean	±SD
Communication scale score	11.65	±0.32	14.7	±1.9	15.1	±1.3
Social scale score	12.1	±0.70	14.5	±0.1.7	14.8	±2.0
Motor scale score	10.21	±0.55	13.8	±0.50	14.9	±2.8
Statistical analysis	Rachitic autistics Versus Rachitic non autistic		Rachitic autistics Versus Controls		Rachitic non autistics Versus Controls	
t/p	t	p	t	p	t	p
Communication scale	7.87	>0.05	14.12	<0.001*	0.93	>0.05
Social scale	5.89	<0.01*	6.57	<0.01*	0.62	>0.05
Motor scale	17.26	>0.05	9.24	>0.05	2.28	>0.05

t = unpaired t-test was used for statistical comparison
 VABS = Vineland Adaptive Behavior Scales
 P>0.05 = statistically non significant, p<0.01* = statistically highly significant

Table 3: Correlation between measured 25 (OH) vitamin D & total CARS score with other studied psychometric and biological variables in studied rachitic cases:

	Variable	r	p-value
25 (OH) vitamin D	Age(years)	-0.381	<0.05*
	Serum calcium (mg/dl)	0.231	>0.05
	Serum ph(mg/dl)	0.062	>0.05
	Serum Alk.ph(IU/L)	-0.400	<0.05*
	Total VABS score	0.260	>0.05
	Total CARS score	-0.338	<0.05*
Total CARS score	Age(years)	-0.266	>0.05
	Serum calcium (mg/dl)	-0.189	>0.05
	Serum phosphorus (mg/dl)	-0.006	>0.05
	Serum alkalin phosphatase (IU/L)	-0.063	>0.05
	Total VABS score	-0.359	>0.05

N.B. “r” = Spearman Correlation co-efficient CARS: Childhood Autism Rating Scale
 VABS = Vineland Adaptive Behavior Scales
 P>0.05 = Statistically non significant, P<0.05 = Statistically significant

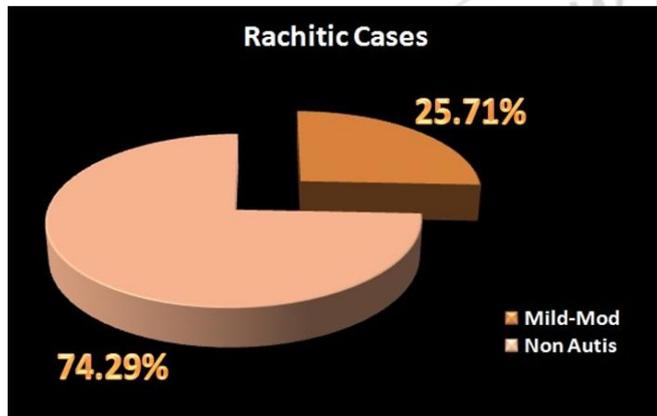


Figure 1: Prevalence of mild to moderate autism encountered among studied vitamin D deficient/insufficient rachitic infants and children

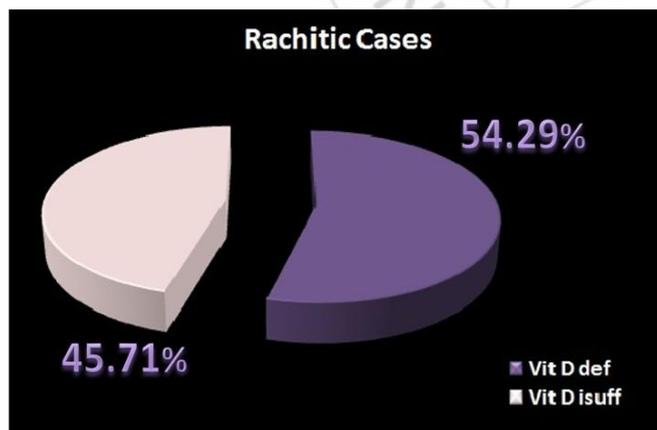


Figure 2: Frequency distribution of vitamin D status categories encountered among studied rachitic infants and children

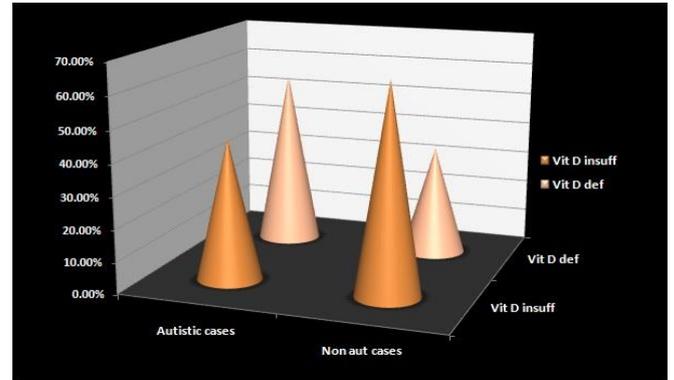


Figure 3: Frequency distribution of vitamin D status categories encountered among studied autistic & non autistic rachitic infants and children

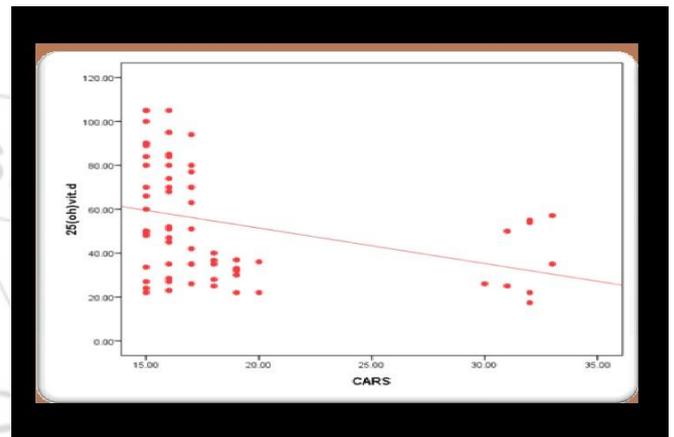


Figure 4: Scattered diagram showing the recorded significant negative correlation between serum 25 (OH) vitamin D and total CARS score in studied cases ($r = -0.338$, $p=0.047^*$)

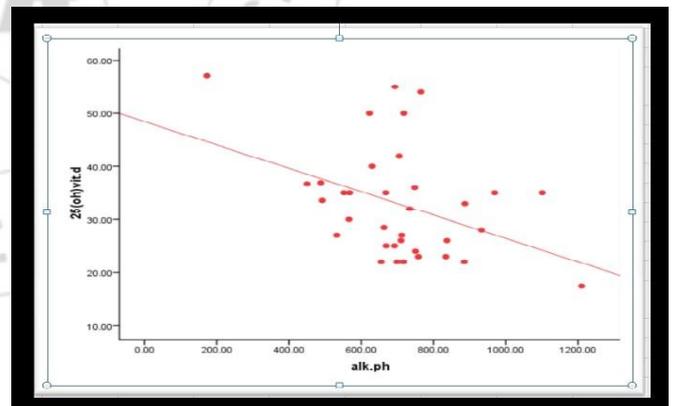


Figure 5: Scattered diagram showing the recorded significant negative correlation between serum 25 (OH) vitamin D and serum alkaline phosphatase in studied cases ($r = -0.400$, $p=0.017^*$)

6. Discussion

Nutritional rickets (NR) is a disease that affects children and adolescents during times of rapid growth [27]. Vitamin D deficiency and/or NR remain prevalent in developing regions of the world, in spite of the sunshine in many of them most of the year, and rank among the 5 most common diseases in children [28]. Prevalence of NR in developed countries on the other hand, appears to be rising [29]. Suggested reasons for its

reemergence include consumption of vitamin D deficient food and changing lifestyles where children spend most of their time indoors on various forms of technology and globalization [30].

On the other hand, it is well known that environmental as well as genetic factors are important in the etiology of autism [31]. **Fernell et al., (2010)** [32] have reported that different environmental factors contributing to vitamin-D deficiency are also associated with increased risk of autism. Vitamin D is crucial for several key physiological processes, including brain development, DNA repair, and regulation of many genes. Much evidence indicates that prenatal and early postnatal vitamin-D deficiency increases autism risk, probably through multiple effects, including impaired brain development and increased de novo mutations [33, 34].

Abdel Nabi et al (2012) [35] found a high incidence of rickets among Egyptian male infants, and their results were in accordance with a previous study of **Elkholy et al., (1992)** [36] who reported that one of the facts of life is the greater susceptibility to disease and to early mortality of the human male. They explained that reasons for these discrepancies between males and females are unknown but might be due to differences between both sexes in development and in genetic constitution. In fact, the majority of studies showed that estrogen, the female hormone, has a positive effect on calcitriol (di OH vitamin D) levels [10]. In the current study, the male to female ratio within the rachitic group was 0.94:1 but this cannot contradict the male sex predilection in cases of rickets taking in consideration that only infants and children whose caregivers agreed to participate in the current study were enrolled. That obligatory condition for study inclusion prevents the objective judgment on that point in our rachitic sample.

The present study revealed that the poor exposure to sunlight with history of parental habitual complete wrapping of children was recorded in 54.29% of enrolled rachitic cases. This finding was supported by **Meguid et al., (2010)** [37] who found lack of exposure to sunlight due to complete wrapping of children to play an important role in the development of NR.

As regards type of feeding, 15 out of 35 studied cases (42.85%) were breast fed without vitamin D supplementation. **Balasubramanian and Ganesh (2008)** [38] & **Ponnapakkam et al., (2008)** [39] revealed that most of the rachitic children were breast fed. Considering the fact that, as breast feeding rates increase, the incidence of vitamin D deficiency rickets is also expected to increase due to the insufficient vitamin D amounts in breast milk. The increase in the practice of breast feeding, associated with the believe that "breast is the best" and it is a baby 's' perfect food" may lead to decrease of 25-hydroxy vitamin D intake from other sources and thereby causing rickets [40].

Delayed motor milestones were found in 22.86% of enrolled cases in the current study. Similarly, **Siddiqui & Rai (2005)**[41] recorded it in 20% of their studied cases.

On the other hand, recurrent respiratory tract infections were found in 37.14% of our studied cases compared to 18.33% of **Siddiqui & Rai (2005)**. They postulated that vitamin D deficiency may predispose to different bacterial infections as 1-25 (OH)₂ D₃ plays an important role in immune modulation. Also, **Yener et al., (1995)** [42] have reported more episodes of bacterial infections in children with vitamin D deficiency. Recurrent diarrhea was found in 22.86% of included patients of the current study and in 20% of **Siddiqui & Rai (2005)** cases. They showed that diarrhea is very common in developing countries due to high prevalence of poor hygiene, non-availability of clean water, and malnutrition. They also reported that these symptoms improved with vitamin D supplementation. **Hameed et al., (1998)** [43] and **Khattak et al., (2004)** [44] have also reported an association of rickets with recurrent or chronic diarrhea.

Chlebowski et al., (2013) [45] supported the use of CARS as a reliable measure for autism severity. **El-Baz et al., (2011)**[46] in an Egyptian study reported that 57% of their studied autistic cases had a severe degree of autism, 28% had a moderate degree, and 15% had a mild degree of it according to CARS Scores. Similar findings were reported by **Bilder et al., (2012)** [47]. In the current study, the mean total CARS score of enrolled rachitic cases was significantly higher than that of controls. On the other hand, mild to moderate autism according to CARS scores and as confirmed by DSM IV TR autistic criteria were encountered in 25.71% of studied cases compared to none of controls; $p < 0.01$. Such finding was consistent with the findings of **Tachimori et al., (2003)** [48] and **Perry et al., (2005)** [49].

The total VABS score was significantly lower in our studied rachitic cases compared to controls. Also, rachitic autistic cases had significantly lower mean communication VABS scale score compared to controls and lower mean social VABS scale score compared to both controls and rachitic non autistic cases. **El-Baz et al., (2011)** [46] reported that 55% of autistic patients presented with mild to severe mental retardation, 36% with below average mentality, and 9% with normal mentality. **Baron-Cohen et al., (2006)** [50] on the other hand, reported that autistic children have spectrum of IQ ranged from 0 to 60.

Studied rachitic cases of the current study had statistically significant reduction of mean values of serum phosphorus and calcium and significant rise of serum alkaline phosphatase compared to controls. Meanwhile, they had significantly lower mean value of 25 (OH) vitamin D compared to controls.. These findings were in accordance with those of **Bakeit and Mageid (2013)** [51] who found a significant difference between rachitic cases and controls as regards serum calcium, phosphorus, alkaline phosphatase, and 25 (OH) vitamin D . Their investigation supported that most rachitic cases had elevation of serum level of alkaline phosphatase, low serum level of 25 (OH) D, calcium and phosphorus deficiency. Similar findings were reported by **Ladhani et al., (2004)** [52] and **Balasubramanian et al., (2008)** [38]. Both serum calcium and phosphorus were known to be sensitive to vitamin D deficiency, referring to a previous study presented by **Schubert et al., (2010)** [53]

who also postulated that hypophosphatemia at vitamin D deficient state was related to muscle weakness of rachitic patients.

Serum alkaline phosphatase generally tends to increase in infancy, therefore its level was less effective to screen vitamin D deficiency in early infancy, but it is a sensitive marker of rickets [54]. In the current study, there was a significant positive correlation between 25 (OH) vitamin D and the serum alkaline phosphatase meaning that the lower the 25 (OH) vitamin D, the higher the alkaline phosphatase. **Bakeit & Mageid. (2013)** [51] also reported a similar positive correlation between the level of alkaline phosphatase and vitamin D.

Many studies suggested that a minimum circulating level of 25 (OH) D should be more than 30 ng/ml. **Holick & Chen (2008)** [55] reported that vitamin D deficiency is considered at levels of < 20 ng/ml and vitamin D inadequacy at levels between 20 and 29 ng/ml. **Choi et al., (2013)** [56] reported that the mean serum level of 25 (OH) vitamin D in their total tested rachitic subjects was 20.15 ± 13.14 ng/ml that was very close to the cut-off value for vitamin D deficiency. The proportion of their cases with serum level of 25 (OH) D < 20 ng/ml was 48.7%. 25 (OH) vitamin D was found to be insufficient in 45.71% and deficient in 54.29% of our studied rachitic cases compared to none of controls. Vitamin D deficiency in early life affects neuronal differentiation, axonal connectivity, dopamine ontogeny, and brain structure and function. Some investigators reported reduced serum 25-hydroxy vitamin D in autistic children. These studies could classify them as being 'vitamin D inadequate', which lends support to the hypothesis that autism is a vitamin D deficiency or insufficiency disorder, respectively [57]. In the current study, deficient and insufficient 25(OH) vitamin D level were recorded in 55.56%, 44.44% of autistic rachitic cases respectively compared to 34.62%, 65.38% of non autistic ones respectively.

25 (OH) vitamin D was significantly negatively correlated with total CARS score of our studied rachitic cases meaning that the lower the 25 (OH) vitamin D, the higher the total CARS score, the severer the autistic manifestations. This finding goes with those of **Mostafa & AL-Ayadhi (2012)** [58] who reported that serum 25-hydroxy vitamin D had a significant negative correlations with CARS and their patients with severe autism had a lower serum 25-hydroxy vitamin D than their studied children with mild to moderate autism but that difference did not reach a statistical significance. The findings of the current study and those of **Mostafa & AL-Ayadhi (2012)** signify a possible link between the extent of vitamin D deficiency and the degree of severity of autism. Both vitamin D receptors and vitamin D metabolizing enzymes are present in CNS. Calcitriol, the active vitamin D, affects numerous neurotransmitters and neurotropic factors, relevant for mental disorders [10, 34].

7. Conclusion

ASDs do occur in vitamin D deficient/insufficient rachitic infants and children. Mild to moderate autism according to

the total CARS score and as proved by DSM IV TR ASDs diagnostic criteria were encountered in 25.71% of the studied vitamin D deficient/insufficient rachitic cases compared to none of controls. Furthermore, 25 (OH) vitamin D has been proven to be significantly negatively correlated with the total CARS scores i.e. the lower the 25 (OH) vitamin D, the higher the total CARS scores, the severe the autistic manifestations.

8. Future Scope

Future studies are recommended on nationwide representative samples to define the nationwide prevalence of ASDs among vitamin D deficient/insufficient rachitic Egyptian children. Such studies will determine the actual magnitude of the problem in our developing country that in spite of being sunny almost all through the year still Egyptian infants and children are showing vitamin D deficiency/insufficiency as well as autism. Meanwhile, it is recommended to be screen for ASDs in vitamin D deficient/insufficient rachitic infants and children as a high risk group for the development of such disorders and to treat them as prompt as possible with proper doses of vitamin D which could represent a magic therapeutic modality for a disorder with such complex pathogenesis like autism. On the other hand, it is advisable to biologically screen autistic children for vitamin D deficiency/insufficiency and treat them with proper doses of it whenever deficient or insufficient. Therapeutic trials with vitamin D in cases of ASDs seem worthy to be studied comparing its efficacy with other available therapeutic modalities for this group of complex neurodevelopmental disorders. Lastly, but by no means least, creating public awareness about the importance of vitamin D for both physical and mental health of infants and children and the necessity of reasonable sun exposure with adequate vitamin D supplementation is crucial.

Conflict of Interest: The authors declare no conflict of interest, no financial, and or personal relationships with other people or organizations that could inappropriately influence our study or theirs.

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