Serum Levels of 25-Hydroxy Vitamin D in Autistic Iraqi Children

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Abstract

Current study targeted 200 autistic children in Baghdad governorate from children referred to the Central Pediatric Teaching Hospital, as well as patients who were referred to Dr. Haidar Al Malki Consultant Clinic in Pediatric Neuromedicin from 1 year to 12 years. The results shows that the percentage of males with autism was 84% compared with females was 16% from total patients, age group distribution was found that the age group of 4-6 years is the most frequent group with 59% of the total patients followed by the age group 7-9 years with 28%. The result of vitamin D3 level showed about 15% of them have Severe Deficiency of D3 by mean level 7.6 ng/ml while 50% had vitamin D deficiency at a concentration rate of 14.2 ng/ml, the mild – Moderate Deficiency (Insufficient) category were appear by 25% at concentration rate of 23 ng/ml, while the percentage of optimal level (Sufficient) of D3 in children have autism were 10% by at concentration rate of 32.6 ng/ml, In conclusionthe average vitamin D concentration for all patients was 17.3, which was classified as vitamin D deficiency.

I. INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder which usually develops in early 3 years of life. ASDs are a heterogeneous group of complex biological and neurodevelopment diseases (Moore et al., 2005).

ASD, with an unknown etiology, remains poorly understood and has no specific treatment. The prevalence of ASD has increased over the past decade and is continuously increasing. This condition made its medical management a challenging task. However, it is unclear whether the increase of its prevalence is due to changes in diagnosis or a true increase in proportion of cases (Ashwood et al., 2006).

Autism is a group of lifelong developmental disabilities with multiple genetic and environmental risk factors which is generally not curable. The people with autism suffer from impaired social interaction and problems in verbal and nonverbal communication. In recent years, there has been a remarkable rise in the prevalence of autism. The increase in prevalence of autism may not only due to an improvement in diagnostic techniques and the rise of people's awareness (Siegel et al., 1984). This disease is associated with other neuro-behavior-cognitive disorders. Its symptoms include a wide range of socio-communication problems, intelligent disabilities, speech problems, attention deficit hyperactivity, seizure disorder, fragile X syndrome, or tuberous sclerosis. Moreover, some children may also have various mental health problems such as depression or anxiety (Gillberg, 2010).

Ming et al and Gonzalez et al detected that ASDs often has a multitude of biological features too. These biological features include systemic pathophysiological disturbances such as increasing oxidative stress, mitochondrial dysfunction, and metabolic or immune abnormalities (Chaste & Leboyer, 2012).

The interaction of genetic and environmental parameters in ASD was investigated in recent years. Lack of vitamin D has newly been suggested as a probable environmental risk factor for autism (Coleman & Gillberg, 2012).

Vitamin D deficiency is now recognized as a pandemic problem. Vitamin D deficiency mainly occurs due low sun exposure, while it is the main source of vitamin D for humans. Vitamin D is a hormone produced in the skin. It has a hormone-like effect on all tissues of the body. Vitamin D regulates bone metabolism, calcium absorption, gene expression, cell replication, differentiation, and death. Vitamin D has a pivotal role in prevention of cancers. Vitamin

D has two different compounds including cholecalciferol found mainly in plants, and ergocalciferol found mainly in animals. To start the production of vitamin D, the skin must be exposed to ultraviolet sunlightand at the end develops an active form of vitamin-calcitriol (1,25-(OH)2D3) (Braunschweig et al., 2013).

Sex	No.	%
Male	168	84
Female	32	16
total	200	100

Vitamin D deficiency causes rickets, osteoporosis, osteopenia, and osteomalacia (bone and body pains). Additionally vitamin D deficiency plays a key role in multiple sclerosis, rheumatoid arthritis, many cancers, depression, autism, falls, heart diseases such as congestive heart failure, type 1 diabetes, high blood pressure, fibromyalgia, chronic fatigue syndrome, parkinsonism, bacterial vaginosis, C-sections, chronic headaches, chronic back pain, osteoarthritis, allergies, eczema, melanoma, psoriasis and gum disease. Appropriate levels of vitamin D boost immune system and protect human against colds and flu (Frye et al., 2013). Levels of 25(OH)D are applied to determine the status of vitamin D level in every individual (Meador & Loring, 2013).

It has been suggested that vitamin D hormone plays a role in autism while there is an association between the incidence of autism and low levels of vitamin D (Landgren et al., 2010).).

Vitamin D has a position in neuro-differentiation, gene regulation, embryogenesis, neuro-immunity, and antioxidant and also anti-apoptosis effects (Idring et al., 2014). A relatively small proportion of studies have focused on the status of vitamin D in patients with autism. The present study aimed to further investigate the potential role of vitamin D in autism by measuring the levels of vitamin D [25(OH)D in children with ASD.

II. MATERIALS AND METHODS

2-1- Location, Duration and Data collection of the Study

The blood samples of autistic children were collected during the period march -September 2017 in from children referred to the Central Pediatric Teaching Hospital and Dr. Hider AL- Maliki clinic in Baghdad province / Iraq, under his supervision.. A total of 5 ml venous blood was aspirated from each individual and plan tube for obtain of serum by centerfugation at 5000 rpm for immunological test. The samples were brought to the laboratory in a well-insulated ice box. All subjects completed a questionnaire on age, gender.

2-2- Serum 25(OH) D3 Determined

Serum 25(OH) D3 levels were measured using the new by ELISA assay kit which was designed for the determination in human serum or plasma samples (Eagle Biosciences Inc., MA, USA).

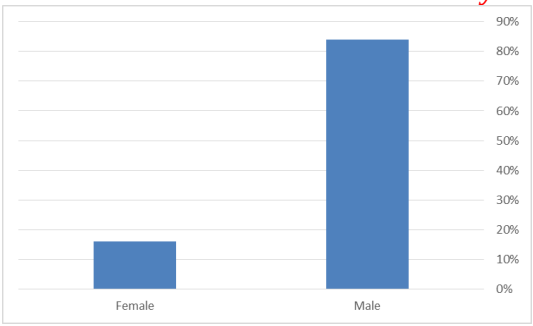
III. RESULTS AND DISCUSSION

Sex with autism

The table 3.1 shows that the percentage of males to females with autism the results showed was 84% in males compared with females, which was 16% from total patients.

Table (1) the percentage of males to females in ASD.

Figure (1) the percentage of males to females in ASD



Boys were at higher risk for autism than girls and approximately the same result reported by Newschaffer et al., (2007), who found that sex ratio was more than 5.5:1, while others like Filipek et al., (1999) found sex ratio was 4:1. Shao et al., (2002) suggested that autism is an X-linked disorder, this may explain male predominant of autism, but others like Hallmayer et al., (1996) , found cases of male-to-male transmission of autism in multiplex families, however, rule out Xlinkage as the predominant mode of inheritance in these families.

In current study, 78.2% of children were males and 21.8% were females. This gender distribution is supported by Sipos et al., (2012) who found that their sample included 73.7% boys, and 26.3% girls that he reported that that autism is five times more likely to occur in boys than in girls.

The male bias in autism spectrum disorder incidence is among the most extreme of all neuropsychiatric disorders. Developmentally males are exposed to high levels of testosterone and its byproduct, estradiol. Together these steroids modify the course of brain development by altering neurogenesis, cell death, migration, differentiation, dendritic and axonal growth, synaptogenesis and synaptic pruning, all of which can be deleteriously impacted during the course of developmental neuropsychiatric disorders. Many neuroanatomical sex differences are established early, beginning in utero and extending to the postnatal period (Sanders SJ et al.,2015). The principle driver is an increase in androgens and estrogens in the brains of developing males as a result of steroidogenes is by the fetal testis. Steroids modulate neurogenesis, synaptogenesis and cell differentiation by inducing or repressing the expression of genes associated with excitation/inhibition, management of calcium and regulators of transcription all of which are dysregulated in ASD (Baron-Cohen , 2010).

There are two sides to the coin of higher rates of ASD in boys. One is the possibility that males carry inherent risk factors that make them more vulnerable to genetic mutation or environmental insult. The other is that girls are inherently protected from the same. Studies that explore a biological origin of the sex difference in ASD emphasize circulating gonadal steroid levels in utero (Turner et al.,2015) or cumulative genetic risk factors that have differential penetrance in boys versus girls (Gockley., 2015). In contrast, several studies support the contention that females carry a higher load of genetic mutation before succumbing to ASD, suggesting they are protected (Robinson et al.,2013) . A third and currently untested possibility is that females are actually more sensitive to genetic anomalies impacting brain development and disproportionately die in utero (Jacquemont S., 2014).

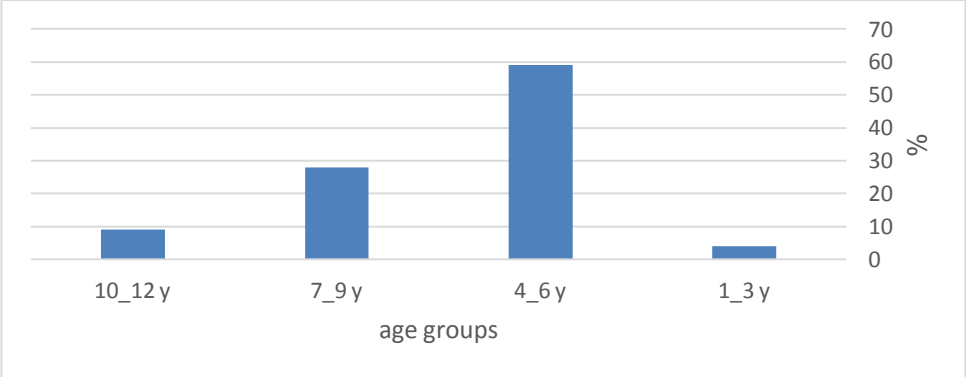
Age groups with autism

The age group distribution, as shown in Table 3-2 it was found that the age group of 4-6 years is the most frequent group with 59% of the total patients followed by the age group 7-9 years with 28%

Table (2) age group distribution in ASD.

Freq.	No.	%
1-3	8	4
4-6	118	59
7-9	56	28
10-12	18	9
total	200	100

Figure (2) age group distribution in ASD.



During the preschool years siblings are generally unable to form a clear picture of ASD, although children as young as three will realize that something is different about their sibling with a disability. The majority of the research on the development of the understanding of illness follows the developmental stages of Piaget's model (Ko et al., 2004)

According to Piaget, children in the preoperational stage understand the world according to their immediate experiences. At this level, personal contact with such experiences are critical for the development of cause and effect. This may help explain why very young children may understand the implications and causes of physical, but not mental disability (Burne et al., 2004).

By three years old, most children have experience some kind of physical illness, which they can later use to relate to the sickness of others. However, for most children a sibling with ASD is the first exposure they have to a person who has a "mental illness." Siblings at this age are therefore likely to create explanations of mental disability that fall into their existing schemas. For example, Becker et al., (2005) asked 3- to 6-year-olds to explain the causes of various non-physical disabilities; most children indicated physical descriptions (e.g., "She fell on her head.").

Children may also overgeneralize their knowledge of physical disability to mental disability. For example, children in one study described both cognitive and physical limitations of children with learning disabilities (Piven et al., 1995). The absence of contagion is also not well understood at this age (Hardan et al., 2001), and preschoolers may worry that they contributed to or can catch their sibling's ASD.

Serum 25-Hydroxy Vitamin D level

Table 3 shows vitamin D status in general. The results of our current study of 200 Iraqi children with autism revealed that about 15% of them have Severe Deficiency of D3 by mean level 7.6 ng/ml while 50% had vitamin D deficiency at a concentration rate of 14.2 ng/ml, the mild – Moderate Deficiency (Insufficient) were appear by 25% at concentration rate of 23 ng/ml, while the percentage of optimal level (Sufficient) of D3 in children have autism were 10% by at concentration rate of 32.6 ng/ml,

The average vitamin D concentration for all patients was 17.3, which was classified as vitamin D deficiency.

Table (3) the range of vitamin D3 level

Serum 25-Hydroxy Vitamin D in ng/ml	Vitamin D Status
≤ 10	Severe Deficiency
10-20	Deficiency
20-30	Mild – Moderate Deficiency
≥ 30	Sufficient
40-50	Ideal
50-150	Indeterminate
>150	Toxicity

Table (4) the main of vitamin D3 level in Iraqi children have autims

State level of Vitamin 25(OH)D in ng/ml	Number Of Participants	%	Mean Level Of Vitamin 25(OH)D
Severe Deficiency <10 ng/ml	30	15	7.6
Deficiency 10-20 ng/ml	100	50	14.2
Suboptimal (Insufficient) 20-30 ng/ml	50	25	23.0
Optimal level (Sufficient) ≥ 30 ng/ml	20	10	32.6
Total	100	100	Average mean 17.3

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 (Holick, 2009). 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 (Vieth, 2006).

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,30 reduced expression of a number of genes involved in neuronal structure, hyperlocomotion, and alterations in both learning and memory (Feron et al., 2005). Furthermore, a French group found that developmental vitamin D deficiency disrupts 36 proteins involved in mammalian brain development (Almeras et al., 2007). Severe gestational vitamin D deficiency in

rats produces pups with increased brain size and enlarged ventricles, anatomical abnormalities similar to those found in autism (Eyles et al., 2003). 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 (Ashwood et al., 2006). Seizures are common in autismand activated vitamin D significantly increases the seizure threshold, making the brain tissue less likely to seize. (Rossi et al., 1995). 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 (Eyles et al., 2005). 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 (Stevens et al., 2000). In the present study, vitamin D levels were lowest during the summer birth season, although no statistical significance was found.

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