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Relation of Vitamin D level with otitis media in autistic children

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Background
Autism spectrum disorder (ASD), is a disorder with a complex clinical presentation and an unknown etiopathogenesis. Immune dysfunction is suggested as a risk factor contributing to co-morbidities observed with ASD. Vitamin D plays a vital immunomodulatory role in bacterial and viral infections besides its newly emerging role in postnatal brain development.
Objective
This study aims to assess the relation between vitamin D and otitis media (OM) in autistic children.OM can present additional challenges to autistic individuals.
Materials and methods
There were 91 participants in the study. They were split into three groups ranging in age from 2 to 12 years. The first group comprised 35 autistic children without OM, the second group comprised 35 autistic children with OM, and the third comprised 21 healthy controls. All 3 groups underwent otoscopy and tympanometry to assess middle ear functions and to investigate the presence of OM, measurement of the vitamin D concentration, CARS-2 for assessment of autism severity, and language assessment to determine their linguistic abilities.
Results and conclusion
Compared to healthy controls, children with ASD had considerably lower serum vitamin D levels. Additionally, children with ASD and OM had notably lower serum Vit D levels than children without ASD. On the other hand, neither the existence of OM nor serum vitamin D levels were correlated with linguistic ability or age.
Serum Vit D level may contribute to the immune modulation in autistic children and lower levels of Vit D might increase the chance of middle ear infections, but it was not correlated with autism severity or language age or verbal communication.

Keywords: Ear infection, otitis media, immune function, pediatric otolaryngology, hearing health, immunomodulation, inflammation.

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Introduction

Otitis media (OM) is an inflammation in the middle ear space where there's a buildup of fluid in it causing middle ear effusion. This effusion is the defining feature of otitis media. When a child feels ill, OM is the most prevalent diagnosis and the most common reason to provide antibiotics [1]. This middle ear effusion is seen in OM with effusion (OME) without any indications of an acute infection. If the fluid persists for three months or more, OME may continue as chronic OM with effusion (COME) [2]. It has been estimated that between 10% and 30% of preschoolers have OME [3]. The estimated prevalence rate for COME is between 5% and 9%. According to estimates, the average incidence of OM-related hearing loss is 30 per 10,000 individuals, making it the main cause of hearing loss in youngsters [4].

A multifaceted medical condition, autism spectrum disorder (ASD) arises from the interplay between environmental and genetic variables. Numerous environmental factors and hundreds of autism risk genes have been identified [5]. Maternal obesity, neonatal hypoxia, folic acid deficiency, and gestational diabetes mellitus are examples of potential environmental variables [6]. Recent

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research indicates that a vitamin D (vit D) deficiency may be a risk factor for autism [7].

Early detection of co-morbidities that accompany autism improves the functioning of autistic children over the long run and helps to understand the shared mechanisms [8]. Due to the compromised cellular and humoral immunity linked to autism, children with autism are more vulnerable to many infections, including OM [9].

Long thought to be primarily related to bone health, vit D is now known to have significant effects on the immune system, such as boosting mucociliary clearance, affecting the synthesis of antimicrobial factors, modifying inflammatory pathways, and affecting microbial communities [10]. Several complications of respiratory disorders, including childhood OM, have been linked to vit D deficiency, according to different studies [11]. Calcitriol, the active form of vit D, is a neuroactive hormone that plays a crucial role in brain development and cognition by promoting neural cell proliferation and neural transmission [12]. Additionally, it is thought that vit D influences the expression of the tryptophan hydroxylase 2 gene (TPH2), which controls the synthesis of serotonin, hence influencing the brain's serotonin production. Executive function and the inhibitory control of impulsive and violent behaviour are impacted by serotonin dysregulation in the brain contributing to an increase in repetitive behaviours and impacting social and commutative functions [13]. Thus, understanding and correlating vit D effect in ASD communicative abilities and behavioural manifestations needs more exploration and elaboration in research.

Several researchers have compared OM and vit D levels in typically developing children [1], in the current study we aim to explore this relation in children with ASD along with studying how Vit D may affect their linguistic abilities.

The purpose of this research is to investigate the association between vit D and otitis media in children with autism.

Materials and methods

Sample collection

This study was carried out to detect the average level of serum vit D in autistic children with and without otitis media in comparison to healthy controls, as there is currently no data available on these differences. In light of the pilot study's findings, comparing the average level of vit D in autistic children with and without otitis media in comparison to healthy controls with a ratio 2:1, and medium effect size (0.69), so a sample size of 21 autistic children with otitis media, 21 autistic children without otitis media, and 11 neurotypical children were required , The 15% compensation was attributed to the utilization of nonparametric tests and made up to 35% of any potential losses so the final sample size was 35 for each group of autistic children with and without otitis media and 21 healthy control to be able to reject the null hypothesis that the population means serum vit D in both autistic children with and without otitis media compared to healthy control are equal with probability (power) 0.95. The test of this null hypothesis has a Type I error probability of 0.05. The G power software was used to calculate the sample size.

This case–control study was carried out at the Autism Spectrum Disorder Clinic and the Audio-Vestibular Clinic, Department of Research on Children with Special Needs, Institute of Medical Research and Clinical Studies. National Research Centre. Cairo, Egypt.

Before beginning the study, informed consent was given to the parent of each subject. There were 91 participants in the study. They were split into three groups as follows: Study group 1: autistic children without OM, consisted of 35 autistic children ages 2 to 12 years. Study group 2: autistic children with OM, consisted of 35 autistic children aged from 2 to 12 years. Study group 3: it consisted of 21 neurotypical children without OM (controls) with ages ranging from 2 to 12 years. The research was not conducted under the following circumstances: fragile x**,** autism associated with syndromes, Children who have a history of vit D supplementation, active rickets, other recognized neurological and psychiatric diseases, and medication formulas that contain vit D as cod liver oil. The children under study had a comprehensive evaluation that comprised taking their medical histories and audiological evaluations such as otologic examinations with otoscopy, and tympanometry (to evaluate middle ear function and OM).

Language assessment using the Arabic Preschool Language Scale for obtaining language age to determine the level of linguistic skills where it is used to acquire both raw and scaled scores of receptive, expressive, and total language abilities [14].

For the diagnosis of ASD, the criteria of Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) for ASD were applied [15] and the Childhood Autism Rating Scale-2 (CARS-2 scale) was used to determine the degree and severity of the ASD symptoms. The scoring at 30 is considered a cut-off rate for diagnosing ASD. Points from 30 to 36.5 are considered autism of mild to moderate degree, and

points from 37 up to 60 indicate autism of severe degree [16].

Serum samples were separated from venous blood for quantitative analysis of 1,25 (OH)2. Prior to liquid extraction of vit D, precipitation of proteins was done by mixing 1 ml of serum with 2 ml acetonitrile. Evaporation of the supernatant to 1 ml volume was done by speedVac concentrator. Liquid extraction was done using ethyl acetate as an organic phase. Five ml of organic solvent were added then vortex mixing for 5 minutes followed by centrifugation.

The supernatant was withdrawn and completely evaporated by a speed vac concentrator. Derivatization was done by adding 150 µl of 4-phenyl-1,2,4-triazoline -3,4 dione (PTAD) with a concentration of 1 mg/ml acetonitrile and incubation for one hour followed by evaporation. The residue was redissolved in 400 µl 40% acetonitrile [17]. Analysis was done on Waters Xevo TQD Triple quadruple tandem mass spectrometer 0.1 % formic acid was used as mobile phase A, while acetonitrile was used as mobile phase B. Gradient elution was done at a flow rate 0.2 ml /min. Starting with 40 % B to 3 minutes then 60 % B at 9 minutes to 10.5 minutes then 90% B at 11 minutes to 14 minutes and back to 40% B from 18 to 25 minutes. Injected sample volume was 10 µl, parent cone voltage was 30 V and collision energy was 15V [18].

Statistical analysis

Datasets were examined with version 6 of GraphPad Prism. To compare the means of the three study groups, one-way ANOVA and post-hoc tests were run. The two ASD groups' quantitative variables were compared using the parametric unpaired t-test. The chi-square test was used to compare the categorical variables. A P-value of less than 0.05 was deemed statistically significant.

Results and discussion

The three studied groups were age and sex-matched, whereas both ASD groups showed comparable language age as shown in Table 1. Based on CARS sub-classification, the two groups included similar proportions of mild-to-moderate and severe cases. The results also showed significantly lower levels of vitamin D compared to controls where ASD with OM had the lowest circulating 1,25-dihydroxyvitamin D concentrations compared to their autistic peers without OM. As shown in Fig.1. No correlation was found between serum Vit D levels and language age in the ASD group Table 2.

Table 1 Comparison of demographic and clinical data.

		ASD with OM (n= 35)	**ASD without OM (n= 35)**	**Control (n= 21)**	***P*-value**
Age (years)		6.3± 2.4	5.6± 2.2	6.9± 2.2	0.12
Languageage(years)		2.8± 1.4	2.0± 1	6.8± 2.4	**<0.0001**[ab]
Gender	Male	23 (65.7%)	26 (74.3%)	11 (52.4%)	0.25
	Female	12 (34.3%)	9 (25.7%)	10 (47.6%)	
ASD	Mild-to-moderate	25 (71.4%)	20 (57.1%)	-	0.21
	Severe	10 (28.6%)	15 (42.9%)	-	
OM	Bilateral Type A	-	35 (100%)	21 (100%)	**<0.0001**[ac]
	Bilateral Type B	31 (88.6%)	-	-	
	Unilateral Type B	4 (11.4%)	-	-	

ASD, autism spectrum disorder; CARS, childhood autism rating scale; OM, otitis media.
Categorical and numerical variables were presented as number (% percentage) and mean± standard deviation, respectively.aControl vs. ASD with OM,bControl vs. ASD without OM, cASD with OM vs. ASD without OM.

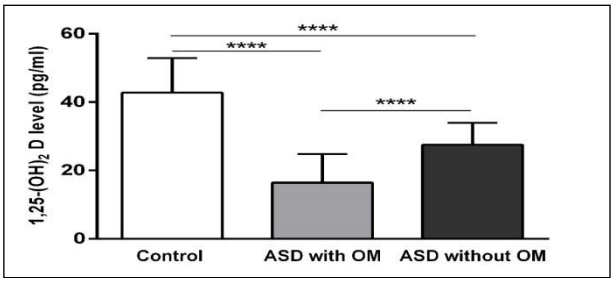

Fig. 1. Serum 1,25-dihydroxyvitamin D levels among study groups.

ASD cases with OM (14.4± 8.4 pg/ml) and without OM (27.5± 6.4 pg/ml) showed significantly lower levels of the vitamin D compared to controls (42.8± 10.1 pg/ml). Children in the former group also exhibited lower circulating 1,25-dihydroxyvitamin D concentrations compared to their autistic peers without OM. Graph was plotted as mean± standard deviation. ****, *P*< 0.0001.

Table 2 Pearson correlation between the language age and serum 1,25-dihydroxyvitamin D levels

	ASD with OM (n= 35)	**ASD without OM (n= 35)**	**Total ASD (n= 70)**	**Control (n=21)**
R	-0.2559	-0.0430	0.08133	-0.4025
P-value	0.14	0.81	0.50	0.07

In contrast to autistic children without OM, the current study aimed to determine the vit D levels in autistic children with this condition. Autistic individuals may have increased rate of infections, including ear infections [19]. Several previous studies reported greater odds of OM in autistic children [20, 21]. In the study by Sabourin et al. [22], autistic children showed a significantly greater occurrence of infection by the ages of one and three years in comparison with controls and even when compared with children having other neurodevelopmental disorder. Additionally, it has been demonstrated that autistic children had a greater rate of ventilation tube insertion [23]. In the present study, both autistic groups had significantly reduced level of vit D than healthy controls. vit D has been reported to exert a crucial effect on brain development, it has been implicated in many processes known to be altered in autism as neuronal differentiation, apoptosis and synaptic plasticity besides its anti-oxidative role [24]. Current literature has extensively investigated vit D status in autistic children [18]. Numerous studies have reported reduced levels of vit D in autistic individuals compared to normal control groups including studies on Egyptian children [25].

Furthermore, results of our study revealed that autistic children with otitis media had significantly lower vit D than the other group without otitis media. A Research has demonstrated the anti-inflammatory effect of vit D [26]. Its ability to control innate and acquired immune responses is one of its main roles. vit D stimulates the generation of antimicrobial peptides by macrophages such as cathelicidin peptides, activates cytotoxic activity of innate lymphoid and natural killer cells which are abundant in mucosal cells as well as decreasing expression of pro-inflammatory cytokines by activated T cells [27]. Moreover, t has been proposed that vitamin D supplementation can be used as an adjuvant therapy because numerous studies have demonstrated that it significantly reduces the incidence of acute OM episodes. [28, 29, 30]. Another study by Saad et al. [31] showed that vit D supplementation increased production of IL-10 and increased count of regulatory T-cells. Other research, however, did not discover a statistically ignificant variation in the average vit D level between children with OM and presumably healthy controls. The authors suggested that the use of vit D as an adjuvant in treatment of OM still needs more exploration [32, 33, 34]. In the current study language assessment was performed on all subjects, assessing the children's linguistic abilities receptively and expressively. The results found significantly lower scores in the ASD group than normal controls which has long been established that children on the autism spectrum show delayed language development than their peers, with fewer semantic knowledge, delayed use of single words, shorter sentences, syntactic and phonological deficits in their sentences. It has long been believed that the spectrum of intellectual difficulties among children with ASD accounts for the fact that up to 20–50% of the autistic population may not develop language abilities appropriately or may become non-verbal [35].

As the Communicative and linguistic deficits in ASD children, are the most concerning symptoms that affect functioning. In the current study, there was no correlation between the verbal ability of the ASD group and serum vitamin D levels. This could be because all the children with ASD had moderate to severe autism and low cognitive abilities. Results from research comparing behavioral outcomes before and after Vit D supplementation in ASD children has been inconclusive [36]. Several studies found non-significant difference between pre and post supplementation [37, 38] and the study by Azzam and colleagues in 2015 found that both supplemented and non-supplemented ASD children, showed improvement after attending consistent behavioral and speech therapy. Although studies by Saad et al. [31] have discovered a link between the intensity of ASD symptoms and serum vitamin D levels. where the most severe cases had the lowest serum vit D level, still these studies encountered some limitations in excluding other factors affecting the improvement, rendering these results as inconclusive in confirming the independent effect of Vit D on behavioral manifestation in ASD.

Conclusion

Serum Vit D levels were found significantly lower in children with ASD compared to normal control. Additionally lower Serum Vit D levels were found in children with ASD and OM, indicating the role of Vit D in immune regulation and risk of middle ear infection. However, serum levels of Vit D didn't seem to have any correlation with language abilities, Autism severity, behavioral and communicative abilities in the group with ASD.

Recommendations

Monitoring children's vit D level as part of routine examinations may be crucial to protecting them from OM and its complications. Any type of infection can have a negative impact on a person's daily routine and ability to collaborate with their care team, especially if they have an ASD diagnosis. It is recommended to further investigate how vitamin D levels may affect different behavioral and communicative symptoms in autism.

Conflicts of interest

The authors declare there are no conflicts of interest.

Authors' contributions

HTS did all the audiological assessment and testing, FH shared with the referral and diagnosis of autistic children, MH: did the lab testing, statistical analysis, WSN: shared with the lab testing, GTO: did all the phonological assessment and testing. All authors have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data, as well as the writing of the manuscript.

Ethical considerations

This study was approved by the ethics committee of Medical Research Ethics Committee, National Research Center with approval number 014100324. The guardians of participants provided written informed consent. Research Ethics Committee – Federal Wide Assurance (FWA). FWA00014747. RHDIRB 2017103002

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