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ORIGINAL ARTICLE

Is serum vitamin-D level is risk factor in pathogenesis of otitis media with effusion

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ABSTRACT

Keywords: Vitamin D; OME; PTH

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Background: Otitis media with effusion (OME) is the most common cause of hearing loss in children, its claimed that Vitamin D deficiency may have a role. **Objectives**: to assess the role of vitamin D deficiency in otitis media with effusion. **Patients and Methods:** this study included 150 children with OME aged 3 to 14 years, Vitamin D level was assessed to all patients compared to Vitamin D level of 50 healthy volunteers. All patients with OME received medical treatment for two weeks and followed up for three months. Then divided into two groups; A1 (120) respond to medical treatment and A2 (30) not respond to medical treatment. Vitamin D levels were compared to these two subgroups and compared to group B (healthy volunteers**). Results:** 135 patients of OME had mild conductive hearing loss , and 15 had moderate conductive hearing loss in comparison to those healthy volunteers with normal hearing with highly statistically significant difference. Both groups of OME had significantly low Vitamin D level in comparison to healthy volunteers. Group A1 the mean Vitamin D level was (11.4 ± 9.8) which is significantly higher than that in group A2 (7.1 ± 5.4).Parathyroid hormone (PTH): was 35.8 ± 19.7 in group A1, (47.8 ± 24.7) in group A2 and (47.8 ± 24.7) in group B with highly statistically significant difference. Alkaline phosphatase (ALP) was 354 ± 198.3 in group A1, (387 ± 174.6) in group A2 and (147 ± 146.3) in group B with highly statistically significant difference. **Conclusion**: OME patients had significantly lower Vitamin D levels with in comparison with normal volunteers which indicate that supplementation of vitamin D may reduce incidence of OME.

INTRODUCTION

OME is a common childhood condition in which there is fluid in the middle ear behind intact tympanic membrane without the signs or symptoms of acute inflammation. [(1)] The common causes of OME are upper respiratory tract infections, enlarged adenoid,allergy, submucosal cleft palate, sinusitis,tumors, and radiation.[(2)]management of OME differs by age of the child and whether they belong to an at-risk group. patients with craniofacial anomalies

as down syndrom, congenital fissure, mental imbalance, range jumble and formative delay was in danger.[3] Many studies support the connection between lack of vitamin D and otitis media with effusion and this may be related to the immune-stimulating effects of vitamin D. but in these studies no adequate information to comprehend the role of lack of vitamin D in the pathophysiology, treatment, protection against OME.[4] All immune system cells, particularly antigen-producing cells like dendritic cells, active T and B lymphocytes, and active macrophages, have been found to have vitamin D receptors. As a result, there is no evidence that children who consume vitamin D may be less susceptible to respiratory tract infections.[5]Vitamin D is an steroid hormone.[6]it has 2 main forms, ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). They are found in foods or supplements; however, vitamin D3 is naturally produced in skin.[7]Vitamin D deficiency may lead to many acute and chronic diseases.[8]Many researches considered this deficiency as a causative or predisposing factor in allergic diseases such as asthma, allergic rhinitis, OME, and BPPV.[9,10,11]The level of vitamin D3 can affect activity of different immune cells It can influence the activity of various immunoglobulins and cytokines, which have a great role in the pathogenesis of allergic diseases. Vitamin D insufficiency in Egypt is endemic, and its more common among females.[10,12] it may be due to different factors variation in sun exposure, clothing, age, obesity, several chronic illnesses. Also there is seasonal variation as vitamin D deficiency especially in season there is less exposure to sun light. A seasonal variation of patients presenting with BPPV has been observed in the United States and Iraq.[13,14]Peoples living in sunny regions, have high serum vitamin D levels due to higher incidence of short wavelengths, ultraviolet B rays from the sun.[14,15]

AIM OF THE WORK

This study aimed to assess the role of vitamin D deficiency in otitis media with effusion.

PATIENTS AND METHODS

In this case control study was applied in the ENT department of Aswan University Hospital, from October 2021 to June 2022.Ethical approval from review board of our college was attained, research code **4/54/7/2020**.

Informed Consent was obtained from parents of children participate in the study after explaining the objectives and steps of the research.

This study group included 150 children with OME aged 3 to 14 years, complete history and clinical examination and otoscopy were done.

Diagnosis of OME based on observation of its clinical signs using Pneumatic otoscopy and confirmed by type B tympanometry up to 3 months after treatment, absent acoustic reflex using tympanometry with evidence of average air bone gap greater than 20 dB using audiometry.

Vitamin D level was assessted to all patients then compared to Vitamin D level in 50 healthy volunteers. All patients with otitis media with effusuion were received medical treatment in the form of antibiotics and mucolytics, anti-inflammatory for two weeks and then followed up for three months. They divided into two groups group A (120) patients who respond to medical treatment and group B (30) patients not respond to medical treatment. Vit D levels were compared between these two subgroups and compared to the healthy volunteers.

Vitamin D status was classified according to measured 25(OH) D concentration: less than 10 ng/mL: deficient; between 11–20: insufficient; higher than 20 ng/ml: optimal .[10]

Patients who didn't respond to medical treatments underwent surgical myringotomy and application of tympanostomy tubes. For myringotomy the outer ear canal was sterilized using

70% alcohol solution, and then myringotomy incision was made at the antero-inferior quadrant of the ear drum by using endoscope.

Exclusion criteria:

- Acute rhinintis and sinusitis,
- Congenital anomalies of the face such as: cleft palate, fractures
- chronic diseases, such as: Allergic rhinitis
- Patients who had a known chronic or systemic disease affecting the level of vitamin D as rickets, renal impairment, liver failure or malignancies
- Diabetes mellitus.
- Vitamin D consumption: patients with history of vitamin D supplementation especially during the last week.
- those whom parents refuse participation in the study

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Tympanometry

Unadulterated tone audiometry(Italian Resonance r27a):Standard unadulterated tone audiometry gives symptomatic data in regards to the degree, type, and setup of hearing misfortune. To air conduction (0.25–8 kHz) and bone conduction (0.5–4 kHz) stimuli, hearing thresholds are determined.

Tympanometry (Italian Resonance r36m): The normal middle ear pressure is between +50 and –150 millimeters of water. During the two seconds of the pressure change described above, the probe tip tone is directed toward the tympanic membrane. Values for normative tympanometry: Adapted from Maico Diagnostics' "The Guide to Tympanometry for Hearing Screening." Ordinarily, pressure is thought of "typical" in the scope of - 150 to +25 (mm water). A consistence top inside these regularizing values recommends an ordinary center ear framework.

Vitamin D assessment

The ELISA (Enzyme-linked Immuno Sorbent Assay) method was used to estimate the serum level of 25 hydroxy vitamin D in children with OME and controls. Based on the idea of competitive binding, the 25-OH Vitamin D total ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA).

Preparation and collection of specimens: The serum that is used in the assay allows for clotting and collects the whole blood through venipuncture. After being intubated for 20 minutes at 37° C, the blood sample can be stored for the long term at 20° C until it is analyzed.

Measure methodology: In order to ensure that all testing conditions were identical, all standards, samples, and controls had been run simultaneously. The necessary number of vials for the Vitamin D release step was obtained. New disposable tips were used to dispense 25 mL of each sample, control, and standard into the vials. In each vial, 50 mL of denaturation buffer was dispensed. At 37 °C, sealed vials were incubated for 30 minutes. 200 µL of Balance Cradle were added to every vial. 50 µL of Compound Form were added to every vial. Each vial contained 50 mL of the enzyme complex. 10 seconds of thorough mixing. In this step, complete mixing of the solution was crucial. The ELISA utilized 200 milliliters of this mixed solution.

ELISA Methodology:

The frame holder contained the desired number of brand-new disposable tips, 200 L of the mixed solution for each Standard, Control, and sample were transferred to the appropriate wells. After careful sealing, the wells were incubated at 37 °C for 60 minutes. The wells' contents were vigorously shaken out. The wells were rinsed four times with 300 milliliters of

diluted Wash Solution. To get rid of any remaining droplets, the wells were hit hard with absorbent paper. 200 µL of Substrate Arrangement were addedto each well. at room temperature, incubated for fifteen minutes. Each well was treated with 100 L of Stop Solution to halt the enzymatic reaction. Using a microtiter plate reader, the absorbance (OD) of each well was determined at 450 nm.

Assessment of Parathyroid hormone(PTH) and Alkaline phosphates

serum alkaline phosphatase , PTH and phosphatase serum level measured by (Beckman Coulter AU analyzer).

Preparation and collection of specimens: The serum that is used in the assay allows for clotting and collects the whole blood through venipuncture. After being intubated for 20 minutes at 37° C, the blood sample can be stored for the long term at 20° C until it is analyzed.

Data analysis using statistical methods:

The data were transferred to a personal computer and examined using IBM SPSS version 20.0 (Armonk, New York: IBM Corporation) Numbers and percentages were used to depict subjective data. The range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR) were used to describe the quantitative data. The distribution's normality was confirmed with the help of the Kolmogorov-Smirnov test. The findings' level of significance was determined to be
< 0.05.

RESULTS

In comparing demographic data between both groups of OME and that of healthy volunteers: The mean age of group A1 is (3.84 ± 2.2) and in groupA2 (4.21 ± 2.11), and in group B was (5.26 ± 3.11) with no significant difference (p > 0.05)(table 1).

Also as regarding sex no significant difference between the three studied groups.

Table (1): comparison between demographic data the two groups of OME and the group of healthy volunteers

History data	**OME respond to medical Treatment (GroupA1) (n = 120)**		**OME not respond to medical Treatment (Group A2) (n = 30)**		**Healthy volunteers (Group B) (n = 50)**		**Test of Sig.**	**p**
Age (years)								
Range.	3 – 8		3 – 8		3– 11		t= 1.62	0.11
Mean ± SD.	3.84 ± 2.2		4.21 ± 2.11		5.26 ± 3.11			
Sex	No.	%	No.	%	No.	%	χ^2= 0.53	0.46
Male	85	69.8	20	66.7	40	80.0		
Female	35	29.2	10	33.3	10	20.0		

As regard clinical data of both groups of OME (Retracted TM,Bulging TM,Adenotonsillar hyperatrophy,URTI,Hearing loss,Ventilation tube insertion).group A1 had 30 patients with retracted tympanic membren, 90 patients with bulging TM, 40 patients with adenotonsiller hypertrophy, and all of them had mild conductive hearing loss. Group A2 had 25 patients with retracted tympanic membrane, 5 patients with bulging TM, 27 patients with adenotonsiller hypertrophy, and 30 patients of them had mild conductive hearing loss (table 2).

Table (2): Comparison between both groups of OME as regard clinical data

Clinical data	Group A1 (N-120)	Group A2(N-30)
Retracted TM	30	25
Bulging TM	90	5
Adenotonsillarhyperatrophy	40	27
Hearing loss	120	30
Ventilation tube insertion	0	30

As regard Audiological assessment, Pure-Tone Audiometry in those patients with OME about 130 patients has mild conductive hearing loss , and 15 with moderate conductive hearing loss in comparison to those healthy volunteers with normal hearing with highly statistically significant difference (table 3).

Table (3): Comparison between both groups of OME as regard

Audiological assessment

Audiological assessment		Cases Group (n = 150)		Control Group (n = 50)		χ^2	P
		No.	%	No.	%		
Pure-Tone Audiometry	Normal Hearing Sensitivity	0	0.0	50	100.0	**150.0**	**<0.001***
	Mild Hearing loss	135	90.0	0	0.0		
	Moderate Hearing loss	15	10.0	0	0.0		
Tympanometry	Type A	0	0.0	50	100.0	**150.0**	**<0.001***
	Type B	150	100.0	0	0.0		

Both groups of OME had significantly low Vitamin D level in comparison to healthy volunteers. in group A1 the mean Vitamin D level was (11.4 ± 9.8) which is significantly higher than that in group A2 (7.1 ± 5.4) which not respond to medical treatment (table 4).

As regard Parathyroid hormone(PTH): it was 35.8 ± 19.7 in group A1, (47.8 ± 24.7) in group A2 and (47.8 ± 24.7) in group B with highly statistically significant difference (table 4).

As regard alkaline phosphatase(ALP) it was 354 ± 198.3 in group A1, (387 ± 174.6)in group A2 and (147 ± 146.3) in group B with highly statistically significant difference (table 4).

Table (4): Comparison between the two studied groups according to Laboratory Investigations

Laboratory Investigations	Medical Treatment(A1) (n = 120)	Surgically treated(A2) (n = 30)	Control Group (B) (n = 50)	Test of Sig.	P
vitamin D(25-OH Vitamin D) (ng/ml) Mean ± SD.	11.4 ± 9.8	7.1 ± 5.4	29.2 ± 13.9	**t= 2.01**	**0.001***
Parathyroid hormone(PTH)(pg/mL) Mean ± SD.	35.8 ± 19.7	47.8 ± 24.7	27.2 ± 14.2	**t= 1.92**	**0.008***
Alkaline phosphatase(ALP) (U/L) Mean ± SD.	354 ± 198.3	387 ± 174.6	147 ± 146.3	t= 0.143	**0.001***

Vitamin D status was classified according to D concentration of measured 25(OH): deficient less than 10 ng/mL; between 11–20: insufficient; higher than 20 ng/ml: optimal (10) in this study; there was highly significant difference between group A1, A2 and control group B as regards Serum vitamin D level ($p < 0.001$) table (5).

Table (5): Classification of serum Vitamin D level in each group into normal, insufficient and deficient

Serum vitamin D level	Medical Treatment (Group A1) (n = 120)		Surgically treated group (Group A2) (n = 30)		Control Group (n = 50) (GroupB)		χ^2	P
	No.	%	No.	%	No.	%		
Normal(above 20 ng/mL)	20	16.7	4	13.3	35	70.0		
Abnormal							**89.27**	**<0.001***
1-Insufficiency(12-20 ng/mL)	45	37.5	9	30.0	10	20.0		
2-Deficiency (12 ng/mL and below)	55	45.8	17	56.7	5	10.0		

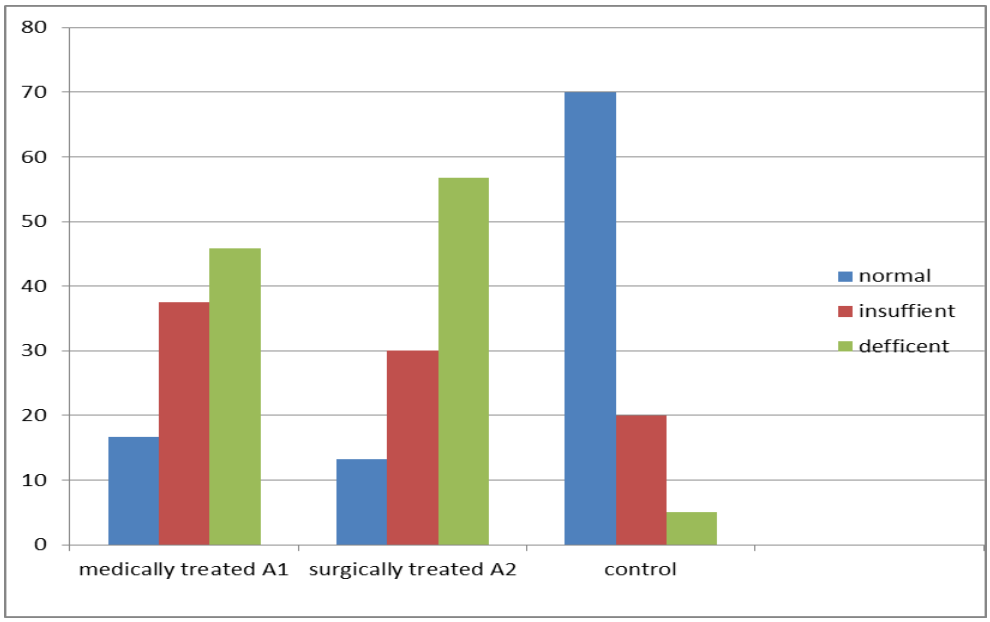

Figure (1): Comparison between Serum vitamin D level in cases and controls

DISCUSSION

Multiple studies have been conducted to correlate human diseases and vitamin D deficiency, especially in otolaryngology, number of ear diseases are claimed to be related to vitamin D deficiencies.[16]

Vitamin D insufficiency has been linked to the onset and recurrence of Meniere's .[17], disease BPPV .[18]. Other studies reported that vitamin D insufficiency may be correlated with middle ear diseases as tympanosclerosisand otosclerosis.[19]

In this study, there is no statistically significant difference between the studied groups in terms of age or sex. this is near to results of **Mohammed et al [20]** .

In this study, audiological assessment with PTA revealed that 90 percent had mild conductive hearing loss, 10 percent had moderate hearing loss, and all patients had

tympanometry type B. In the control group audiological assessment with PTA revealed that all patients had normal hearing and tympanometry type A which is the same results of**Mohammed et al [20].**

Hosseini et al found that all of their patients had tympanometry type B, with 95 percent of them having mild conductive hearing loss and 5 percent having moderate hearing loss which is also near to results of this study**[21]**.

This study reported that the mean serum vitamin D level in children with OME group A1 was 11.4 ± 9.8ng/ml versus 7.1 ± 5.4ng/ml in group A2, and in the healthy controls it was 29.2 ± 13.9ng/ml.

In study done by Mohammed et al 2021,the mean vitamin D level was 17.02±8.49 ng/m in patients of OMEl. In comparison to control group, the mean vitamin D level was 25.85±8.94 ng/ml with *highly* statistically significant difference.(20) Also in study done by Cayir et al results were matching with this study. , they performed a case–control study and comparing vitamin D levels in children with recurrent OME and healthy volunteers. the mean vitamin D level was 11.4±9.8 ng/ml in patients with OME in comparison to control group (29.2±13.9) ng/ml.[20]

Additionally, contrary to study done byHosseini et al the mean level of vitamin D in children with OME was 26.1±14.6 ng/ml and in children in the control group, it was 29.5±17.9 ng/ml with no statistically significant difference.[21].[21]Additionally, our findings are consistent with those of **Akcan et al** who compare the serum vitamin D levels of children with OME and control children and found significant difference between the study group and the control group **[22]**.

In this study the mean degree of PTH in patients with OME group A1 was 35.8 ± 19.7ng/ml and in group A2 was 47.8±24.7ng/ml while in group B it was 27.2±14.2ng/ml with P value (0.008).**Cayir et al**determined that there was a statistically significant difference in terms of PTH between the patients with OME and the healthy controls**[23]**.

In this study group A1 there was (16.7%) of patients were typical vitamin D level, (37.5%) were lacking and (45.8%) were inadequate. group A2 13.3%of patients were ordinary vitamin D level, (30%) were deficient and (56.7%) were inadequate.group B there were (70%) of the healthy volunteers were ordinary,(20%) were deficient and (10%) were lacking with significant difference.

Matching our result to that of **Cayir et al.[23]**who reported that31% of patients with OME have ordinary degrees of vitamin D, 19% have inadequate degrees of vitamin D, and 50% have insufficient degrees of vitamin D. While in the control group; 70% have typical degrees of vitamin D, 26% have lacking degrees of vitamin D, and 4% have insufficient degrees of vitamin D. with statistically significant difference between the two groups**.[23]**.Hosseini et al Also found 25 % of patients with OME had normal vitamin D levels, 55% had insufficient vitamin D levels, and 20% had deficient vitamin D levels

Asghari *et al.* reported lower serum vitamin D level in patients of OME. The mean level vitamin D in the OME patients was 9.79±4.36 ng/ml and in the healthy group, it was 13.61±6.33 ng/ml and with *statistically significant difference*. also They reported in OME patients; 6.2% have normal vitamin D levels of, 21.9% have insufficient vitamin D levels, and 76.8% have deficient vitamin D levels. While in the healthy volunteers, 31% have normal vitamin D levels, 33.3% have insufficient vitamin D levels, and 35.7% have deficient vitamin D levels with significant difference between the two groups..**[24]**

But they found no significant difference significant differences between vitamin D and adenotonsillar hypertrophy with and without OME [24, 25]. Also in meta-analysis study done by Li et al, in 2016 concerning of relation between vitamin D and incidence of otitis media;the results showed a relation between the development of recurrent AOM and vitamin D deficiency, but no significant relation between OME and serum vitamin D deficiency [25,26].

CONCLUSIONS

1- generally Children with OME had significantly lower level of serum Vitamin D than normal volunteers
2- patients with OME who didn't respond to medical treatment have significantly lower serum levels of 25-hydroxy vitamin D than children who respond to medicaltreatment.
3- This suggests that vitamin D deficiency may have a role in OME
4- Vitamin D supplementation may reduce the incidence of OME.
5- more studies are needed to confirm the relation between vitamin D deficiency in OME in children.

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