

## Vitamin D levels in children diagnosed with acute otitis media

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### Abstract

**Objective:** To investigate the relationship between Vitamin D deficiency and acute otitis media infection.

**Methods:** The randomised, single-blind, case-control study was conducted at the Paediatric Department of Ataturk University, Erzurum, Turkey, from January to April 2010. It comprised ambulatory children diagnosed with acute otitis media and healthy controls. The subjects were divided into groups according to their serum 25-hydroxy vitamin D levels. SPSS 18 was used for statistical analysis.

**Results:** Of the 169 subjects in the study, 88(52%) were the cases and 81(48%) were controls. The mean age of the cases was  $6.21 \pm 3.4$  years, and  $6.18 \pm 3.12$  years for the controls ( $p < 0.951$ ). Serum 25-hydroxy vitamin D levels in the cases and controls were  $20.6 \pm 10.2$  ng/mL and  $23.8 \pm 10.3$  ng/mL ( $p < 0.05$ ). There was no statistically significant difference between the groups in terms of parathormone and calcium levels ( $p > 0.05$ ).

**Conclusion:** Serum 25-hydroxy vitamin D levels being significantly lower in children diagnosed with acute otitis media compared to the controls in two otherwise similar groups suggests that Vitamin D deficiency plays a role in otitis media infection.

**Keywords:** Acute otitis media, Child, Vitamin D. (JPMA 64: 1274; 2014)

### Introduction

Otitis media (OM) is inflammation of the middle ear and the most commonly seen infection in children after upper respiratory tract infections.<sup>1</sup> Since upper respiratory tract infection attacks (viral or bacterial) increase the risk of acute OM (AOM), the risk of AOM rises in winter when upper respiratory tract infections are common. A rise in the incidence of OM depends on a combination of several factors, and particularly conditions in which sensitivity to Eustachian tube dysfunction and recurrent respiratory tract infection rises.<sup>1,2</sup>

The presence of Vitamin D receptors in several tissues suggests that Vitamin D may play a role in the etiopathology of diseases.<sup>3-5</sup> Vitamin D receptors have been described in all immune system cells, particularly cells producing antigens, such as active T and B lymphocytes, active macrophages and dendritic cells. Monocytes and macrophages' chemotactic and phagocytic properties have been shown to rise in an environment in which Vitamin D is present and that their microbicidal characteristics are therefore strengthened, and attention has turned to the role of Vitamin D in immune regulation.<sup>4,6-8</sup> Several studies have reported a

correlation in children between low or inadequate Vitamin D concentrations and respiratory tract infections.<sup>9-17</sup>

The purpose of the current study was to investigate the relationship between Vitamin D levels and AOM.

### Patients and Methods

The randomised, single-blind, case-control study was conducted at the Paediatric Department of Ataturk University, Erzurum, Turkey, from January to April 2010 after obtaining approval from the institutional ethics committee and consent from the parents of all the children in the study.

The study group comprised ambulatory children who were diagnosed with AOM, and healthy controls. The sample size was calculated using Raosoft sample size calculator using 5% margin of error, confidence level of 95%, and response distribution of 5%. Patients were selected by random sampling. First, all children diagnosed with AOM were consecutively assigned to one group, and healthy children assigned to the control group. The children between 1 and 13 years of age who were free of any craniofacial abnormality, chronic diseases and acquired or congenital immunodeficiency were prospectively included. AOM was diagnosed on the basis of 3-element criterion: acute onset, inflammatory signs or symptoms (otalgia, severe tympanic hyperaemia), and signs of occupation of the middle ear or otorrhea.<sup>18</sup> Physicians documented visit records that included the identification number of the subject, the date of the visit, and at least one diagnosis. The child's symptoms and

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baseline characteristics were recorded, and a clinical examination, including thorough pneumatic otoscopic examinations, was performed. In the subjects in whom otoscopic examination presented inconsistency or ambivalence, visual otoscopy was re-performed by another paediatrician in a blind manner. In case of

## Results

Of the 169 subjects in the study, 88(52%) were the cases and 81(48%) were controls. The mean age of the cases was  $6.21 \pm 3.4$  years, and  $6.18 \pm 3.12$  years for the controls ( $p=0.951$ ). Besides, 55(62.5 %) of the AOM group were boys and 33(37.5 %) girls, compared to 52(64.2 %) boys

**Table:** Comparison of the AOM and control group vitamin D levels.

Groups	Vitamin D levels			Total (n/%)	P value
	Normal (n/%)	Insufficiency (n/%)	Deficiency (n/%)		
AOM	38 (43.2%)	18 (20.5%)	32 (36.4%)	88 (100%)	0.004*
Control	64.2 (66.7%)	17 (21%)	12 (14.8%)	81 (100%)	

Not The Pearson Chi square test was performed. n, number of individuals; AOM, Acute otitis media  
\* $P < 0.05$  was regarded as significant.

AOM: Acute otitis media.

continuing the inconsistency or ambivalence, the subject was excluded from the study.

The case and control groups were matched for age and gender. The controls were admitted to the paediatric outpatient clinic for reasons other than systemic problems. Controls were similar to the cases except for AOM.

All records of the cases were reviewed and details were: age of onset; laboratory parameters that included Parathormone (PTH), Serum 25-hydroxy (25[OH]) vitamin D, Calcium (Ca), Phosphorus (P), and Alkaline phosphatase (ALP). Serum 25(OH)D levels above 20ng/mL were regarded as normal, while levels between 15-20ng/mL were considered vitamin D insufficient, and levels <15ng/mL were categorised as vitamin D deficient.<sup>5</sup>

Blood samples were taken immediately after the diagnosis of OM. All blood samples were stored at  $-40^{\circ}\text{C}$  until analysis. All the tests were performed according to the manufacturer's instructions. Serum Ca, P, ALP levels were determined using a Roche Cobas 8000 System (Tokyo, Japan) along with Roche Diagnostics kits. Levels of 25(OH)D were determined in an E-170 enhanced chemiluminescence (ECL) system (Roche, Japan) with an electrochemiluminescence method. PTH was measured by chemiluminescent enzyme immunoassay, IMMULITE (DPC Co. USA) autoanalyser.

All parameters except for serum P and ALP levels had normal distribution pattern. The data was subjected to independent sample T test (for normally distributed parameters). Mann Whitney test (for skewed parameters) and Pearson's chi-square tests using SPSS 18. Significance was declared at  $p < 0.05$ . Results were expressed as mean  $\pm$  standard deviation.

and 29(35.8 %) girls in the control group ( $p=0.819$ ).

The difference between the 25(OH)D levels in the AOM and control groups was statistically significant (Table).

Mean PTH level in the AOM group was  $42.07 \pm 14.9$  and  $41.44 \pm 14.41$  in the control group ( $p < 0.739$ ). AOM and control group mean Ca, P and ALP values were  $9.39 \pm 0.57$  and  $9.52 \pm 0.84$ ;  $5.08 \pm 0.7$  and  $4.84 \pm 0.79$ ; and  $283.5 \pm 69.1$  and  $223.5 \pm 81.7$ , respectively. In terms of Ca, there was no statistically significant difference between the groups significant (95% confidence interval[CI]: 0.34-0.09;  $p < 0.261$ ). The differences between the groups in terms of serum P and ALP were statistically significant ( $p < 0.001$ ).

## Discussion

Since upper respiratory infection attacks (viral or bacterial) increase the risk of AOM, the risk of OM rises in winter when upper respiratory tract infections are frequently seen. AOM agents frequently originate from colonising bacteria in the nasopharynx. The most important of these are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. No agent can be isolated in 10-40% of AOM cases. Viral agents are thought to be involved in these. Important viral agents include syncytial virus, rhinovirus, adenovirus and influenza virus. life-threatening complications can result when appropriate treatment is not given.<sup>18-20</sup>

The difference in incidence seen in children with similar sociodemographic characteristics points to individual sensitivity. The fact that we determined a difference in 25(OH) D levels between the cases and the controls, despite the families having similar characteristics, there being no difference between the groups in terms of exposure to sunlight and that children were not receiving

regular Vitamin D support, suggests that there is a correlation between OM and Vitamin D.

Active Vitamin D receptors have been described in many tissues, including the hypophyses, ovaries, skin, stomach, pancreas, thymus, breast, kidney, parathyroid glands and lymphocytes. These studies show that Vitamin D has different functions in addition to calcium metabolism.<sup>4,6,21,22</sup> There is a powerful correlation between Vitamin D and both natural and acquired immunity. Anti-microbial peptides (defensin, cathelicidin) involved in natural immunity and reactive oxygen products cause the death of micro-organisms. Active Vitamin D stimulates the synthesis of antimicrobial peptide-cathelicidin from natural killer cells and respiratory tract epithelial cells, in addition to epithelioid and myeloid cell series.<sup>23-26</sup> Calprotectin and S100 proteins, important natural immune system regulators, also increase under the effect of active Vitamin D.<sup>23</sup>

When an infection develops in the epidermis, the Toll-like receptor (TLR) in keratinocytes is stimulated and cathelicidin is expressed. In this way, the organism is to some extent protected against environmental pathogens through the interaction of Vitamin D and the natural immune system. Similarly, the active Vitamin D manufactured locally in macrophages contributes to the localisation of infection by permitting the release of some cytokines from T lymphocytes and immunoglobulins from active B lymphocytes.<sup>4,23,24,27</sup> Monocytes and macrophages have been shown to increase their chemotactic and phagocytic characteristics in an environment containing Vitamin D, and their microbicidal properties are thus strengthened. In addition to their antigen providing features in acquired immunity, monocytes and macrophages are known to play a key role in the activation of natural immunity against the invasive characteristics of various infections.<sup>4,23,28</sup>

In the event of Vitamin D deficiency, pro-inflammatory cytokines (Interferon gamma [IFN-Gamma], Interleukin 2 [IL-2], tumour necrosis factor alpha [TNF-Alpha]) increase in association with the response to the more powerful Tianhe-I (Th1), immune response is impaired, leukocyte chemotaxis is affected and the predisposition to infection rises.<sup>13,23</sup> The first prototype infection pertinent to the relationship between Vitamin D deficiency and infections is tuberculosis. Studies over the last 20 years have determined a powerful correlation between decreased serum 25(OH) D levels and sensitivity to tuberculosis infection and the severity of that infection.<sup>24,29,30</sup>

Invasive pneumococcal infections, meningococcal infections and group A streptococcal diseases are

frequently seen in Vitamin D deficiency, especially in winter months. These three bacteria are sensitive to microbicidals induced by Vitamin D. Vitamin D deficiency has been shown to establish a predisposition to upper and lower respiratory tract infection and tonsillitis.<sup>10,28</sup> A study<sup>28</sup> showed decreased cathelicidin synthesis in the bronchial epithelial cells in patients experiencing frequent respiratory tract infections and suggested that inhaler Vitamin D could be used to increase cathelicidin synthesis. Some studies have also shown that Vitamin D is effective as an adjuvant therapy in the treatment of several infections.<sup>16,17</sup>

## Conclusion

The findings support the idea that Vitamin D has an important immunoregulatory role and that the incidences of infections may rise when Vitamin D is lacking. Vitamin D deficiency can prepare the ground for frequent infections such as OM. Further prospective studies are needed to reveal the efficacy of Vitamin D as an adjuvant in the treatment of OM.

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