

Some Pro and Anti-Inflammatory Cytokines in Children with Tonsillitis and their Correlations with Vitamin D Deficiency

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Abstract

Inflammation of the tonsils could be described as acute tonsillitis, mainly due to infection. Recurrent tonsillitis could be defined as 3-7 episodes during the first 3 years of age. Vitamin D, which is a neuro-hormone with pleiotropic biological activities may modulate the immune response by alleviation, and stimulation of Th1 and Th2 cell proliferation, respectively, that influence the stimulation, synthesis, and secretion of both pro and anti-inflammatory cytokines. In this study we aimed to shed light on the levels of vitamin D in children with different episodes of tonsillitis in association with levels of interleukins (TNF α , IL-2, IL-4, IL-10). Blood samples were collected from 48 participants in 3 groups: control, acute tonsillitis (1-2 episodes/year), chronic tonsillitis (more than 7 episodes/year), serum was separated and the levels of Vitamin D, TNF α , IL-2, IL-4 and IL-10 were estimated using ELISA technique. Vitamin D decreased significantly as the episodes of tonsillitis increased, with level of 16.38 ± 2.41 ng/ml in acute and, 14.13 ± 2.15 ng/ml in chronic tonsillitis as compared to control (30.91 ± 2.31 ng/ml), while pro-inflammatory cytokines (TNF α and IL-2) significantly increased (46.88 ± 14.05 and 44.55 ± 9.24 , 1267.25 ± 111.85 and 1191.72 ± 121.52 ng/ml, respectively) as compared to control (9.45 and 138.48 ng/ml respectively). Anti-inflammatory (IL-4, IL-10) cytokines in control group were (243.08 ± 28.72 and 24.27 ± 1.83 ng/ml, respectively), which increased non-significantly in acute and chronic tonsillitis (302.76 ± 38.93 , 290.12 ± 44.69 and 28.16 ± 2.01 , 26.29 ± 1.99 ng/ml, respectively). Significant direct correlation was observed between the levels of vitamin D and anti-inflammatory cytokines in chronic tonsillitis ($P < 0.05$). In conclusion, deficiency of vitamin D may affect the number of episodes of tonsillitis in children by modulation of the secretion of some cytokines.

Keywords: Tonsillitis, Vitamin D, Inflammatory markers

بعض السايوتوكينات المحفزة والمضادة للالتهاب لدى الاطفال المصابين بالتهاب اللوزتين وارتباطها مع نقص فيتامين د

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الخلاصة

التهاب اللوزتين او كما يوصف بالتهاب اللوزتين الحاد والذي يحدث على الغالب بسبب العدوى. التهاب اللوزتين المتكرر يعرف بانه ٧-٣ نوبات خلال الثلاث سنوات الاولى من العمر. فيتامين D هو هورمون عصبي له فعاليات بيولوجية متعددة الجوانب ممكن ان يحور الاستجابة المناعية من خلال تخفيف وتنشيط تزايد خلايا Th1 و Th2 على التوالي والذي يؤثر على تنشيط تركيب وافراز كل من السايوتوكينات المحفزة والمضادة للالتهاب. في هذه الدراسة هدفتنا الى تسليط الضوء على مستويات فيتامين D لدى الاطفال الذين لديهم عدد نوبات مختلفة من التهاب اللوزتين وربطها مع مستويات الانترلوكينات (TNF α , IL2, IL-4, IL-10). تم جمع عينات الدم من ٤٨ مشارك بثلاث مجاميع وهم مجموعة الكونترول ومجموعة التهاب اللوزتين الحاد (٢-١ نوبة في السنة) ومجموعة التهاب اللوزتين المتكرر (اكثر من ٧ نوبات في السنة). تم فصل مصل الدم وتم قياس مستويات كل من فيتامين D, TNF α , IL2, IL-4, IL-10 باستخدام تقنية الاليزا. لوحظ ان فيتامين D يتناقص بصورة معنوية كلما ازدادت عدد نوبات التهاب اللوزتين كمستوى ($16,38 \pm 2,41$ نانو غرام / مليلتر) في الالتهاب الحاد و ($14,13 \pm 2,15$ نانو غرام / مليلتر) في الالتهاب المتكرر مقارنة بالكونترول ($30,91 \pm 2,31$ نانو غرام / مليلتر). بينما السايوتوكينات المحفزة للالتهاب (IL2, TNF α) تزداد بصورة معنوية ($46,88 \pm 14,05$, $44,55 \pm 9,24$ و $1267,25 \pm 111,85$ و $1191,72 \pm 121,52$ نانو غرام / مليلتر بالتتابع) مقارنة بالكونترول ($9,45$ و $138,48$ نانو غرام / مليلتر بالتتابع).

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السايوتوكينات المضادة للالتهاب وهم (IL4 و IL10) في مجموعة الكونترول كانت (٢٨,٧٢±٢٤٣,٠٨ و ١,٨٣±٢٤,٢٧ نانو غرام / مليلتر بالتتابع) والتي تزداد بصورة غير معنوية في التهاب اللوزتين الحاد والمزمن (٣٨,٩٣±٣٠٢,٧٦ و ٤٤,٦٩±٢٩٠,١٢ مقابل ٢,٠١±٢٨,١٦ و ١,٩٩±٢٦,٢٩ ng/ml بالتتابع) لوحظ ان هناك ترابط معنوي مباشر بين مستويات فيتامين د وبين السايوتوكينات المضادة للالتهاب في حالة التهاب اللوزتين المتكرر ($P<0.05$). كخلاصة ان النقص في فيتامين D ممكن ان يؤثر على عدد نوبات التهاب اللوزتين لدى الاطفال من خلال تحويل افراز بعض السايوتوكينات.

الكلمات المفتاحية: التهاب اللوزتين، فيتامين D ، علامات الالتهاب

Introduction

Inflammation of the tonsils could be described as acute tonsillitis, mainly contributed to infection. It ranges from localized tonsil infection to generalized infection of the pharynx, as part of the spectrum of pharyngitis. It may affect both sexes at all ages, but mostly it affects younger people, especially in winter season⁽¹⁾. Viruses may account to 50-80% of the causative pathogens, as influenza, herpes simplex, Epstein-Barr virus (EBV), and rhinovirus^(2,3). The most common group of bacteria that could be associated with tonsillitis is Group A beta-haemolytic streptococci (GAS), which cause 5-36% of cases⁽⁴⁾. *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*, are other types of bacteria that can invade the pharynx and tonsils⁽⁵⁾. Fungi species such as *Candida* can cause sore throat in immune-compromised patients⁽⁶⁾.

Seven tonsillitis episodes in one year or five tonsillitis episodes in the previous two years and more or three tonsillitis episodes in the previous three years and more, could be defined as recurrent tonsillitis^(7,8). Development of recurrent tonsillitis, could be associated with many factors, these are including patient non-compliance, premature cessation of anti-biotherapy, bacterial tolerance, bacterial load, bacterial biofilms, inadequate antibiotic absorbance, and deficiencies in the immune system⁽⁹⁾.

Two chemical compounds - ergosterol, or vitamin D₂, and cholecalciferol, or vitamin D₃ could be associated with the term "vitamin D". Active vitamin D₃ (calcitriol) is considered as a neuro-hormone because of its pleiotropic biological activities⁽¹⁰⁾. In spite of the growing knowledge about the relation between calcium-phosphorus metabolism and vitamin D (VD) and many other roles of vitamin D plays, data suggest that immune cells and a large number of tissue cells, express enzymes that metabolize vitamin D, providing a biologically acceptable mechanism to convert local, auto and paracrine of the native circulating forms, into the active vitamin D₃ (calcitriol)⁽¹¹⁾. Vitamin D immune-modulation includes alleviation, and stimulation of Th1 and Th2 cell proliferation, respectively. Primarily, Vitamin D₃'s suppression of Th1 development is assumed to be mediated by its influence on APC, which reduces IL-2 synthesis, whilst increased expression of Th2 specific transcription factors (GATA-3 and C-Maf) is linked to increased Th2 cytokines production⁽¹²⁾. Correspondingly, vitamin D can stimulate or prohibit the synthesis, secretion, and release of anti-

inflammatory interleukins (IL-10 and IL-4) and pro-inflammatory cytokines (IL-1, IL-2, tumor necrosis factor Alpha (TNF- α), interferon Gama (IFN- γ)), respectively^(11,13). As a result, vitamin D appears to reduce inflammation while allowing for a targeted immune response (In other words, bioactive vitamin D may boost innate immunity while suppressing adaptive immunity). This process is essential for normal function of the immune system, and subsequently inadequate or scanty levels of vitamin D could lead to impairment of immune responses⁽¹⁴⁾.

In this study, we aimed to shed light on the levels of vitamin D in children with different episodes of tonsillitis in association with levels of interleukins (IL-2, TNF- α , IL-4, and IL-10) .

Subjects and Methods

This study was carried out among 48 children between 2 and 7 years of age (mean rang 5.79±0.63) at Al-Yarmook teaching hospital - Otolaryngology department in Baghdad during the period November 2018 and March 2019. Children were classified into 3 groups: group 1(control) which includes 16 children with no tonsillitis problem (mean age 7.00 ± 0.7), second group (group 2): include 16 children with mean age (5.62 ± 0.65) and 1-2 episodes per year (acute tonsillitis), while group 3(chronic): include 16 children (mean age 6.25 ± 0.95) who have recurrent tonsillitis with more than 7 episodes per year.

Tonsillitis was diagnosed depending on physical examination by specialist physician. Frequency of tonsillitis in a year is checked by referring the feedback of parents (by asking the parents). The inclusion criteria for the study groups were as follow: children having one or more episode of tonsillitis/year, having symptoms of tonsillitis which developed within last 5 days, not taking antibiotics therapy in the past week and have no vitamin D supplementation. While the exclusion criteria include children with chronic or systemic diseases, condition requiring hospitalization, children taking immunosuppressant drugs and those with previous operations.

Blood samples were collected centrifuged to separate serum from the three groups. Serum levels of 25(OH) D (supplied by CalBiotech, cat# 220B), TNF- α (Elabscience, cat. No.E-EL-H0109), IL-2 (Elabscience, cat. No.E-EI-H0099), IL-4 (Elabscience, cat. No.E-EI-H0101) and IL-10 (Elabscience, cat. No.E-EI-H0103) were estimated using enzyme linked immunosorbent assay (ELISA) technique and according to the manufacturer.

The study was carried out according to the approval of the research ethical committee in the College of Pharmacy, University of Baghdad. The statistical analysis system- SAS (2012) program was used to compare between control and patient groups in study parameters. Student T- test was used to significantly compare between means, (0.05 and 0.01 probabilities). Also the correlation coefficient between variables in this study has been investigated.

Results

Serum levels of different parameters were measured using ELISA technique in all studied groups and the results revealed a significant decrease (P<0.05) in Vitamin D level in both acute

and chronic group (16.38 and 14.13 ng/ml respectively) as compared to the control group (30.91 ng/ml) (Table 1) (Figure 1). The levels of pro-inflammatory cytokines, TNFα and IL-2 appears to be increased significantly (P<0.05) in patient's groups (46.88, 44.55 and 1267.25, 1191.72 ng/ml, respectively) as compared to the control group (9.45 and 138.48 ng/ml respectively) (Table 1) (Figure 2 and 3). An increase in the level of interleukin 4 and 10 was also observed in the both acute and chronic groups (302.76, 290.12 and 28.16, 26.29 ng/ml, respectively) as compared to the control group (243.08 and 24.27 ng/ml, respectively), yet this increase was not significant (P>0.05) (Table 1) (Figure 4 and 5).

Table 1. levels of Vitamin D, TNFα, IL2, IL4 and IL10 (ng/ml) in different studied groups*

	Vitamin D	TNFα	IL2	IL4	IL10
Control	30.91± 2.31	9.45± 0.918	138.48± 12.09	243.08± 28.72	24.27± 1.83
Acute	16.38± 2.41	46.88± 14.05	1267.25± 111.85	302.76± 38.93	28.16± 2.01
Chronic	14.13± 2.15	44.55± 9.24	1191.72± 121.52	290.12± 44.69	26.29± 1.99
P value/control-acute	0.0001**	0.012**	0.000**	0.227	0.164
Pvalue/control-chronic	0.000**	0.000**	0.000**	0.382	0.462
Pvalue/acute-chronic	0.49	0.891	0.6507	0.83	0.514

*all values represent mean±SE

**Differences are significant when P<0.05

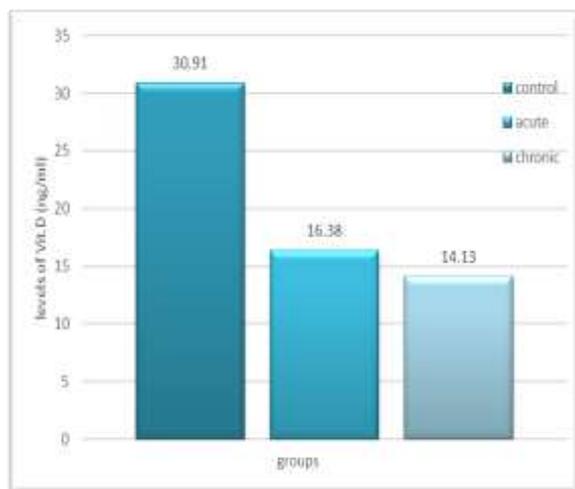


Figure 1. level of Vitamin D (ng/ml) in different groups

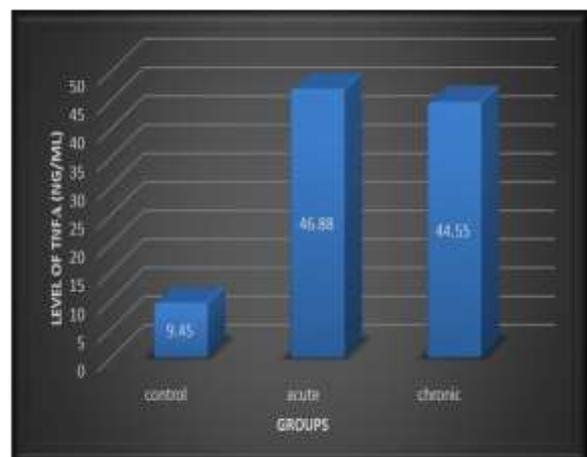


Figure 2. level of TNFα (ng/ml) in different groups

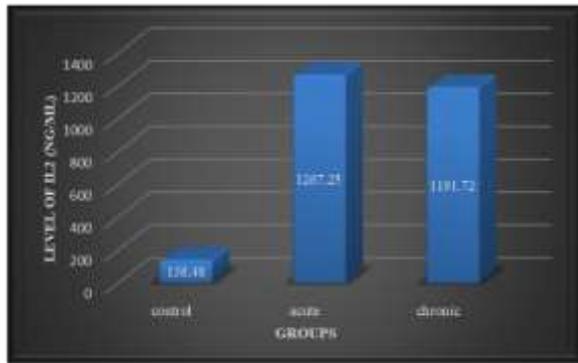


Figure 3. Level of IL-2 (ng/ml) in different groups

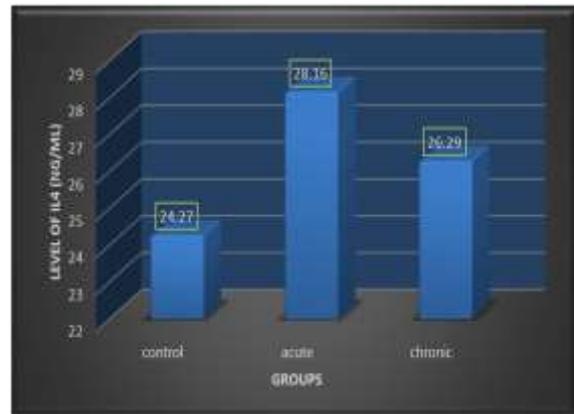


Figure 5. level of IL-10 (ng/ml) in different groups

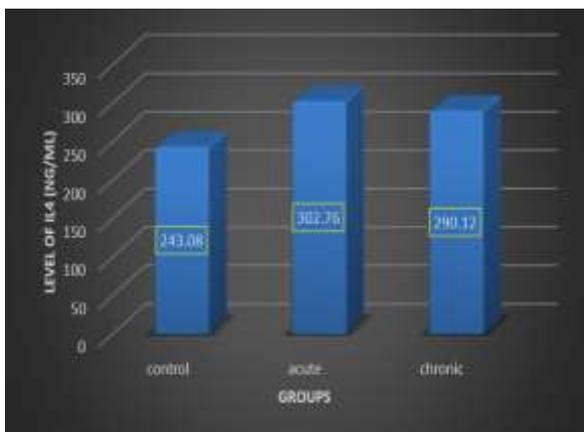


Figure 4. Level of IL4 (ng/ml) in different groups

This study, also aimed to find the correlation between the levels of Vit.D and levels of both, pro and anti-inflammatory cytokines. Vitamin D showed non-significant ($P>0.05$) inverse correlation with pro-inflammatory cytokines ($TNF\alpha$ and IL-2) in all groups. Anti-inflammatory cytokines (IL-4 and IL-10) showed non-significant ($P>0.05$) positive correlation in both, control and acute tonsillitis, and positive significant ($P<0.05$) correlation in chronic group (table 2) (Fig. 6 and 7)

Table 2. correlation coefficient between Vitamin D levels and pro-inflammatory cytokines (IL-2 and $TNF\alpha$) and anti-inflammatory cytokines (IL-4 and IL-10) in different groups.

		IL-10 control	IL-4 control	IL-2 control	TNF control
Vitamin D control	Pearson Correlation	0.179	0.402	-0.152	-0.126
	Sig. (2-tailed)	0.508	0.123	0.573	0.642
	No	16	16	16	16
Vitamin D acute	Pearson Correlation	0.261	0.423	-0.344	-0.107
	Sig. (2-tailed)	0.329	0.103	0.191	0.692
	No	16	16	16	16
Vitamin D chronic	Pearson Correlation	0.764**	0.713**	-0.147	-0.416
	Sig. (2-tailed)	0.001	0.002	0.586	0.109
	No	16	16	16	16

No: number of samples

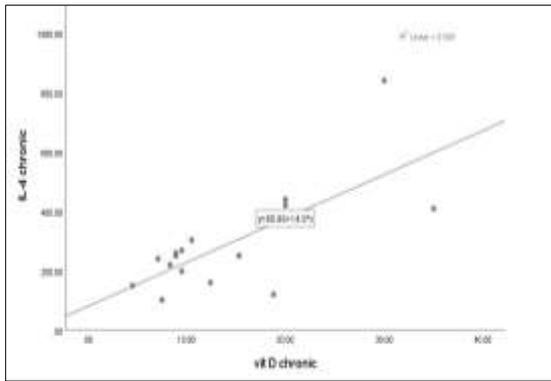


Figure 6. Vitamin D in correlation to anti-inflammatory cytokine IL4 in chronic group

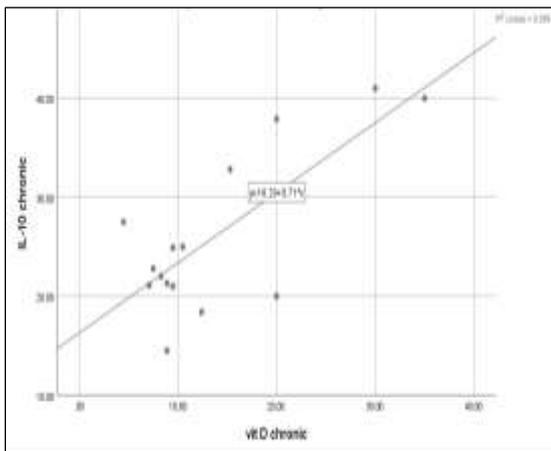


Figure 7. Vitamin D in correlation to anti-inflammatory cytokine IL-10 in chronic group

Discussion

Tonsillopharyngitis consider as one of the leading causes of childhood hospital visits and is characterized as acute tonsil and pharynx infections. The most common causes in etiology are viruses and Group A Beta-hemolytic streptococci ⁽¹⁾

Vitamin D deficiency is associated with an increased frequency of upper respiratory tract infections. (16), According to our results there were significantly lower levels of vitamin D in acute and chronic tonsillitis groups comparing to the control. Similarly, in the previous studies vitamin D levels were significantly lower in tonsillitis patients as compared to the healthy people suggesting that low vitamin D levels may be significant in the pathogenesis of tonsillar diseases ^(9, 17)

The immune responses to the infection are regulated by a balance that stimulates the synthesis of cytokines for Th1 and Th2. The Th1-derived cytokines (IL-2 and TNF- α) induce a response through a cell-mediated immunity. While humoral reaction is induced by Th2, involving secretion of IL-4, IL-5, IL-6, IL-10, IL-15, IL-16 ⁽¹⁸⁾.

It has become quite important to study interleukins as mediators of the immune system that regulate the immunity of mucous membranes.

Chronic inflammatory infectious diseases of the respiratory tract may involve cytokines as mediator of specific immune response and non-specific effector mechanism. Moreover, cytokines may assess the clinical course, magnitude and clinical significance of the pathological process ⁽¹⁹⁾. In this study, the levels of some pro-inflammatory cytokines (TNF α and IL-2) and anti-inflammatory cytokines (IL-4 and IL-10) in tonsillitis patients and healthy (control) groups has been measured.

Pro-inflammatory cytokine TNF α , has a vital role in the host defense against parasitic, viral and bacterial pathogens, especially against intracellular bacterial infections ⁽²⁰⁾. In this study TNF α was significantly increased in acute and chronic tonsillitis groups compared to control. These results are consistent with the study of Aysha and colleges who showed that patients with chronic and acute tonsillitis have significantly higher TNF α than the control ⁽²¹⁾. The reason is that TNF- α activates the vascular lining and increases vascular permeability, increasing immune cell entry, complement proteins, and Immunoglobulin, including IgG and IgA. It also increases fluid discharge to the lymph nodes leading to swollen tonsils ⁽²²⁾.

Interleukin 2 (IL-2) is pro-inflammatory cytokine secreted by Th1 cells and activates T cells to produce TNF α and IFN γ ^(23, 24). The cytolytic function of natural killer (NK) cells can also increase by IL2, thus ensuring their importance in the immune function, and effectually participate in the pathogenesis of multiple pathological situations like infectious, metabolic, cancer, autoimmune and inflammatory diseases ⁽²⁴⁾. According to our study patients with acute and chronic tonsillitis have significantly higher IL-2 than the control group.

Regulation of inflammation could be mediated by some anti-inflammatory cytokines for example IL-4, some of which directed at influencing the activity of macrophage. IL-4 inhibit pro-inflammatory cytokines (TNF α , IL-12, and IL-1 β) and chemokines (IFN γ -inducible protein 10 and macrophage inhibitory factor) ⁽²⁵⁾. Our results showed that IL-4 non significantly increase in both chronic and acute tonsillitis as compared to control. Similar results obtained by Sydney *et al.* who showed that, in both children with acute pharyngotonsillitis induced by B-hemolytic streptococci and control groups, levels of anti-inflammatory cytokines, particularly IL-4, were identical, indicating an alteration in the immune response regulated by Th1 ⁽²⁶⁾. Another study shows that the production of Th1 type cytokines (TNF α and IFN- γ) was elevated in recurrent tonsillitis as compared to Th2 type cytokines production (IL-4 and IL-6), suggesting the influence of the cellular (Th1 type) immune response with respect to the humoral (Th2 type) immune response ⁽²²⁾.

Monocyte, T regulatory cells, NK, dendritic cells (DCs) and macrophages, are the main immune cells that produce anti-inflammatory cytokine IL-10⁽²⁰⁾. Various anti-inflammatory functions by IL-10 and its role as a major regulator of non-specific immunity had been reported⁽²⁷⁻²⁹⁾. In our study, IL-10 non significantly increase in both acute and chronic tonsillitis compared to the control group, because in tonsillitis production of cytokines start with Th-1 type including TNF α and IFN- γ and later on secretion of Th-2 cytokines⁽²²⁾.

There are many lines of evidence to support vitamin D's possible role in improving the balance of anti-inflammatory cytokines. These include studies in which vitamin D has been shown to increase the levels of anti-inflammatory cytokines including beta 1 transforming factor (TGF β 1) and interleukin IL4⁽³⁰⁾ and to inhibit secretion of the pro-inflammatory ones (IL-6, IFN- γ , IL-2, and TNF α)⁽³¹⁾. It has also been shown that vitamin D is important for normal macrophage functions, while its deficiency has been linked to impaired chemotaxis, phagocytosis,⁽³²⁾ and induce inflammation via up regulation of toll-like receptors, on macrophage cell surface⁽³³⁾.

This study investigates the correlation between vitamin D level and inflammatory cytokines in children with tonsillitis. According to our results vitamin D have non-significant negative correlation with pro-inflammatory cytokines (TNF α and IL-2) and positive non-significant correlation with anti-inflammatory cytokines (IL-4 and IL-10) in both control and acute tonsillitis groups, while in chronic tonsillitis vitamin D have significant positive correlation with anti-inflammatory cytokines and non-significant negative correlation with pro-inflammatory cytokines. We conclude that deficiency of vitamin D may affect the number of episodes of tonsillitis in children by modulation of the secretion of some cytokines. The correlation between vitamin D administration and serum levels of inflammatory markers such as cytokines has been investigated previously^(34 - 36). Some studies have indicated that there is a direct correlation with IFN γ and IL-10⁽³⁴⁾, while a significant inverse correlation with serum TNF α , and no correlation with serum IL-10 or IL-6 has been reported⁽³⁵⁾. Some other studies indicate that alteration in the serum levels of pro- and anti-inflammatory cytokines was non-significant⁽³⁶⁾.

This study has several limitations first; CRP level which is considered as best indicator of inflammation was not measured for the studied groups in which low levels of CRP exclude the inflammation despite the healthy appearance for the control group (i.e the healthy children may have inflammation due to any causes despite the healthy appearance) Second; cytokines have short peripheral half-life and should preferably be "locally" assessed, i.e. in tonsils⁽³⁷⁾. Third; children in the studied

groups may have deficiency in other trace elements and vitamins that may affect on the function of the immune system and cytokines production, such as, selenium, zinc, copper, iron, folic acid, vitamins A, E, C, and B6 which are of important significance for response of the immune system⁽³⁸⁾. Fourth; this study investigate the immunological response in tonsillitis patients and its correlation with vitamin D in general regardless of it causes whether virus or bacteria (each one of them may have its immune response), so as recommendation for further studies its better to measure the correlation between vitamin D and inflammatory markers in different types of pathogens.

Conclusion

In this study, the levels of vitamin D were significantly lower, in acute and chronic tonsillitis children. In both groups of tonsillitis, Th-1 cytokines production (TNF α and IL-2) were higher in comparison to Th-2 cytokines (IL-4 and IL-10). Vitamin D has non-significant inverse correlation with pro-inflammatory cytokines and non-significant positive correlation with anti-inflammatory cytokines in both control and acute tonsillitis groups; while in chronic tonsillitis vitamin D have significant positive correlation with anti-inflammatory cytokines and inverse non-significant correlation with pro-inflammatory cytokines. Thus, deficiency of vitamin D may affect the number of episodes of tonsillitis in children by modulation of the secretion of some cytokines. Further studies are needed to evaluate the correlation between vitamin D and other inflammatory markers in patients with tonsillopharyngitis and whether vitamin D replacement therapy can prevent the recurrent tonsillitis.

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