Available online **www.ijpras.com**

International Journal of Pharmaceutical Research & Allied Sciences, 2016, 5(1):180-190

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Evaluation of Serum Vitamin D, LL37 and Interferon Gamma Levels in Saudi Children with Acute Lower Respiratory Tract Infection

Wael Mansy[1,9]* Enas A. Zakaria[2], Somaya EL-Gawhary[3], Sarah S. Alsubaie[4], Manal M. Abouelkheir[5], Amal Fatani[6], Fadwa Abd El Reheem[3], Heba El Awady[7]Nermin H. Ibrahim[8]

[1]*Clinical Pharmacy Department, College of Pharmacy, King Saud University, KSA*
[2]*Pharmaceutics Department, College of Pharmacy, King Saud University, KSA*
[3]*Clinical Pathology Department, College of Medicine, Fayoum University, Egypt*
[4]*Pediatric Infectious Diseases unit, King Saud University Medical City, King Saud University, KSA*
[5]*Pediatric Clinical Pharmacy Services, King Saud University Medical City, King Saud University, KSA*
[6] *Pharmacology and Toxicology Department, College of Pharmacy, King Saud University, KSA*
[7]*Pediatrics Department, College of Medicine, Fayoum University, Egypt*
[8]*Medical Microbiology and Immunology Department, College of Medicine, BeniSuef University, Egypt*
[9]*Pharmacology Department, Faculty of Medicine, Cairo University, Egypt*
*Corresponding Author: Wael Mansy (whsayed@hotmail.com)

ABSTRACT

Background: *Since vitamin D deficiency is prevalent problem among Saudi population and hence its immunomodulatory role is increasingly investigated, this study tries to link between the immunomodulatory role of vitamin D and the risk of LRTI in children.* ***Objectives:*** *Evaluation of serum vitamin D, plasma LL37, and serum interferon γ levels in pediatric patients with acute lower respiratory tract infection.* ***Material and methods:*** *Fifty children with ALRTI admitted at King Khaled University Hospital, Riyadh/KSA were enrolled in the study. Seventy-three apparently healthy children were used as a control group. The results were compared with a matching group of apparently healthy children. We aimed to assess the correlations among serum vitamin D levels, inflammatory markers, and infection status.* ***Results:*** *We found that there was a high prevalence of vitamin D deficiency among Saudi children in general, regardless of the presence of ALRTI. No significant differences in serum vitamin D or plasma LL37 levels were observed between groups. In contrast, serum interferon-γ was found to be significantly higher in children with ALRTI. Moreover, patients with serum vitamin D deficiency were found to have longer hospital stays than patients with normal serum vitamin D levels [P < 0.001].* ***Conclusions:*** *These findings support the immunomodulatory role of vitamin D in the clinical course of ALRTI.*
Key Words: *Vitamin D deficiency, ALRTI, Plasma LL37*

INTRODUCTION

Active vitamin D (1, 25 dihydroxy vitamin D) is a fat-soluble vitamin that plays a major role in bone metabolism and calcium homeostasis and is also a steroid hormone with a variety of other functions [1].

The main source of vitamin D in humans is *in vivo* biosynthesis. The inactive form (25-hydroxy vitamin D) derived from the skin or diet is the major circulating form and is the form that is measured when determining a person's serum vitamin D status. Inactive 25-hydroxyvitamin D is acted upon in the liver by 1, 25-α-hydroxylase, resulting in the formation of active 1, 25 dihydroxy vitamin D, which is metabolized in the kidney to its biologically active form, 1, 25-dihydroxy vitamin D3 [2] .

Vitamin D has well-known roles in calcium homeostasis and bone mineralization [3]. Additionally, vitamin D increases serum calcium concentrations through mobilization of calcium from bone secondary to its deficiency in serum [4]. Additionally, within the last 50 years, vitamin D has been shown to act as both a vitamin and a prohormone [5].

In the last decade, vitamin D deficiency has re-emerged as a serious health problem among individuals in the United States of America [6]. Few studies have evaluated the prevalence of vitamin D among Saudi populations in different geographical regions in Saudi Arabia. One study evaluated vitamin D levels in Dammam/KSA and showed that there is a high prevalence of vitamin D deficiency in middle aged Saudi Arabians living in the Eastern region of the country [7].

A variety of factors can cause serum vitamin D deficiency. Many factors, including dark skin, affect a person's risk of vitamin D deficiency. Typically, dark-skinned individuals require up to six times longer exposure to sun rays to synthesize the same amount of vitamin D synthesized by fair-skinned individuals [2]. Additionally, in older individuals (i.e., over 70 years of age), the skin produces 70% less vitamin D than the skin of younger individuals. In obese individuals, vitamin D is stored in fat cells rather than activated in the liver, as is observed in normal-sized people. Accordingly, obesity and vitamin D deficiency are commonly associated [2].

Vitamin D produced *in vivo* had been shown to contribute to innate immunity. Patients suffering from active tuberculosis exhibit a high prevalence of vitamin D deficiency (86%) [8]. In patients in the intensive care unit, vitamin D deficiency is associated with high mortality rates [8].

Innate immunity plays a major role in the defense against invading pathogens, including bacteria and viruses [9]. There are two major types of cationic antimicrobial peptides (CAMPs), i.e., cathelicidins and defensins (α and β). Both are considered important members of the innate immunity mechanism in humans [10]. Cathelicidins are the only type of human CAMP and are approximately 18 kDa in size (designated hCAP18). Upon activation, hCAP18 is changed to another protein molecule with 37 amino acids and 2 Leucines at the N-terminal (designated LL37) [11]. LL37 acts through local chemo attraction by white blood cells, particularly neutrophils and monocytes; moreover, LL37 exhibits bactericidal activities and can stimulate the proliferation and recovery of injured epithelium [12]. Other studies have suggested that LL37 functions in killing bacteria either through disruption of the cell membrane, dysfunction of the mitochondrial membrane, or inhibition of cell wall or protein synthesis [13].

Because of its role in innate immunity, LL37 is increased in patients with respiratory tract infection [15]. Moreover, patients on chronic hemodialysis with high levels of LL37 were found to be more likely to complete kidney dialysis for one year without acquiring a fatal infection [14]. The antimicrobial effects of LL37 involve the prevention of biofilm formation [17]. LL37 is thought to cause break down of *Pseudomonas sinusitis* biofilm and to prevent biofilm formation [15]. Additionally, cathelicidin has been shown to exhibit strong effects on the immune responses against *Mycobacterium* [during the clinical course of tuberculosis] [2], enteric infections [19], and skin infections [16].

In this context, vitamin D has been shown to have stimulatory effects on LL37 secretion [17]. Additionally, treatment of macrophages with 25-hydroxy vitamin D increases LL37 expression [18]. This may occur through two mechanisms: either increasing the expression of the vitamin D receptors (VDRs), or activation of enzymes that transform inactive 25-hydroxy vitamin D into active 1, 25-dihydroxy vitamin D [18]. Other studies have described vitamin D as an immunomodulator that plays an important role in the regulation of inflammation through affecting cytokines or regulating apoptosis [15].

Furthermore, because the cathelicidin LL37 plays a role in innate immunity in lung infections, this protein is increased in cases of respiratory tract infection [15]. There is a positive relationship between serum vitamin D and LL37 levels [23]. In a study of Ethiopian children with rickets, clinical vitamin D deficiency was found to be associated with a 13-fold increased risk of pneumonia among children less than 5 years of age [19].

Therefore, in this study, we aimed to evaluate the levels of serum vitamin D in young patients [1–10 years old] with acute lower respiratory tract infection (ALRTI) compared with age-matched apparently healthy controls. Moreover,

we evaluated possible association between low serum vitamin D and some immunological markers, including interferon (IFN)-γ and LL37. In addition, we assessed the correlations among serum vitamin D levels, infection type, and ALRTI severity.

SUBJECTS AND METHODS

The current study included 50 patients with ALRTIs with evidence of pneumonia and bronchopneumonia admitted to the Pediatric Department of King Khalid University Hospital [KKUH], King Saud University, Riyadh/ KSA. The patients' ages ranged from 1 to 10 years. Sample collection occurred from November 2014 to March 2015. This study included an age-matched control group of 73 apparently healthy children who were recruited from the vaccination pediatric clinic at KKUH. Written informed consent was obtained from the parents or legal guardians of each participant (patients and controls).

Exclusion criteria:

Patients with underlying respiratory conditions, i.e., aspiration pneumonia or chronic lung disease, were excluded from the analysis. In addition, patients with underlying congenital abnormalities, oropharyngeal anomalies, Down syndrome, or Williams's syndrome, were excluded. Patients with previous history of vitamin D supplementation within the previous 6 months were also excluded.

Analytical methods:

All children were subjected to analysis of serum 25-hydroxycholecalciferol (vitamin D), serum IFN-γ, and plasma LL37 concentrations.

25-Hydroxycholecalciferol was assayed using electrochemiluminescence binding assays (ECLIAs), a type of competitive protein binding assay, c (o) bas®, (Roche). For this kit, 25-hydroxycholecalciferol deficiency is defined as a concentration of 74 nM or less. The preferred level for vitamin D is recommended to be greater than or equal to 75 nM. [20].

Human LL37 was assayed using an LL37 enzyme-linked immunosorbent assay (ELISA) kit (Hycult Biotech HK321; Hycult Biotechnology, Uden, the Netherlands) [21]. LL37 is present in plasma at concentrations ranging from 25 to 250 ng/mL and is enhanced in infectious diseases.

Serum IFN-γ was assayed by ELISA using a human IFN-γ ELISA kit (Merck Millipore; cat. no. EZHIFNG) [22].

Statistical analysis:

Data were statistically described in terms of frequencies [number of cases] and relative frequencies (mean ± standard error of the mean). Comparisons between study groups were carried out using independent sample t-tests. A probability value [*p* value] of less than 0.05 was considered statistically significant. All statistical calculations were performed using IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA).

All experiments were approved by the Institutional Review Board (IRB)/College of Medicine/KSU, under Research Project #E-14-1250 in May 2014.

RESULTS

The current study included 73 apparently healthy children as the control group [age range: 1–10 years, average age: 39.64 ± 3.5 months]. Fifty patients with ALRTI with evidence of pneumonia or bronchopneumonia were also included (average age: 38.06 ± 3.8 months). Viruses, namely respiratory syncytial virus (RSV), caused the majority of cases (86%), whereas only 14% of cases were caused by bacterial infections. Demographic data for both cases and controls are shown in Table 1.

Serum vitamin D levels were measured for all participants. Thirty of the 73 children in the control group [42.5%] had vitamin D deficiency, with average serum vitamin D levels of 50.3 ± 14.6 mg/dl. Moreover, 52.0% (26/50) of children with ALRTI had vitamin D deficiency, with average serum vitamin D levels of 46.1 ± 17.2 mg/dl. Figure 1 shows the prevalence of vitamin D deficiency in the control group and in patients with ALRTI. Table 2 shows the three variables measured in both groups.

Regarding the cause of infection, five of seven patients who had ALRTI due to bacterial infections also had low serum vitamin D levels (71.4%), while only 21 of 43 patients who had ALRTI due to viral infections also had low serum vitamin D levels (48.8%).

Next, we compared serum vitamin D, serum IFN-γ, and plasma LL37 levels among patients and controls. The mean vitamin D level in the control group was 78.18 ± 3.6, which was considered normal, whereas that in patients with ALRTI was 69.82 ± 4.8, indicating deficiency. However, this difference was statistically significant. Patients with ALRTI had no significant differences in their 25-hydroxy vitamin D levels when compared with the control group. For plasma LL37, the mean concentration in the control group was 40.45 ± 4.32, while that in patients with ALRTI was ± 4.44. Again, this difference was not statistically significant. In contrast, serum IFN-γ levels were significantly different between controls and patients with ALRTI. Patients had significantly higher IFN-γ levels (129.44 mg/dl) than controls (89.07 mg/dl) ($p < 0.001$). These results are presented in Table 2 and Figure 2 and 3.

Patients with vitamin D deficiency had significantly lower mean vitamin D levels than patients with normal serum vitamin D. Notably, however, patients with vitamin d deficiency did not have significantly different IFN-γ or plasma LL37 levels than patients with normal vitamin D levels. Thus, the decrease in serum vitamin D was not accompanied by decreased immunological markers. Figure 2 shows serum vitamin D, serum IFN-γ, and plasma LL37 levels in patients with ALRTI with and without vitamin D deficiency.

In order to evaluate the correlation between vitamin D deficiency and the severity of the ALRTI status, we compared the length of hospital stay in patients in the two subgroups according to vitamin D deficiency. Length of stay is a term used to describe the duration of a single episode of hospitalization. Inpatient days are calculated by subtracting the day of admission from the day of discharge [23]. The average length of stay in patients with ALRTI with normal serum vitamin D was 4.37 ± 0.32 days; in contrast, in patients with ALRTI with serum vitamin D deficiency, the average length of stay was 7.08 ± 0.61 days. Figure 5 and Table 3 show the length of stay in patients with ALRTI.

Table 1. Demographic data for patients with ALRTI and apparently healthy controls.

	Controls (n = 73)	**Patients (n = 50)**
Age in months (Mean ± SEM)	39.64 ± 3.5	38.06 ± 3.8
Sex	35 girls 38 boys	25 girls 25 boys
Vitamin D deficiency (%)	42.5%	52%
Cause of ALRTI	----------	Viral: 86% Bacterial: 14%

Table 2. Serum vitamin D, serum IFN-γ, and plasma LL37 levels in controls and patients with ALRTI.

	Controls (means ± SEMs)	**Patients with ALRTI (means± SEMs)**	**t**	***p***
Serum vitamin D	78.18 ± 3.6	69.82 ± 4.83	1.415	0.160
Serum IFN-γ	89.07 ± 1.99	129.44 ± 15.99	3.01	< 0.001
Plasma LL37	40.45 ± 4.32	42 31 ±.4.44	0.291	0.772

Table 3. Levels of vitamin D, interferon-γ, and LL37 and length of hospital stay in patients with ALRTI with or without vitamin D deficiency.

	Patients with ALRTI			
	Normal vitamin D	Vitamin D deficient	t	*p*
Vitamin D	99.7 ± 4.2	42.24 ± 3.0	11.16	< 0.001
Interferon-γ	146.39 ± 30.4	113.79 ± 12.4	1.02	0.31
LL37	44.13 ± 7.9	40.63 ± 4.6	0.39	0.70
Length of stay	4.4 ± 0.32	7.1 ± 0.6	3.81	< 0.001

P< 0.001: significant difference between groups; *p*> 0.05: no significant difference.

Table 4. Levels of vitamin D, and length of hospital stay in patients with ALRTI with or without vitamin D deficiency.

	Patients with ALRTI			
	Normal vitamin D	Vitamin D deficient	t	*p*
Vitamin D	99.7 ± 4.2	42.24 ± 3.0	11.16	< 0.001
Length of Stay	4.4 ± 0.32	7.1 ± 0.6	3.81	< 0.001

P< 0.001: significant difference between groups; *p*> 0.05: no significant difference.

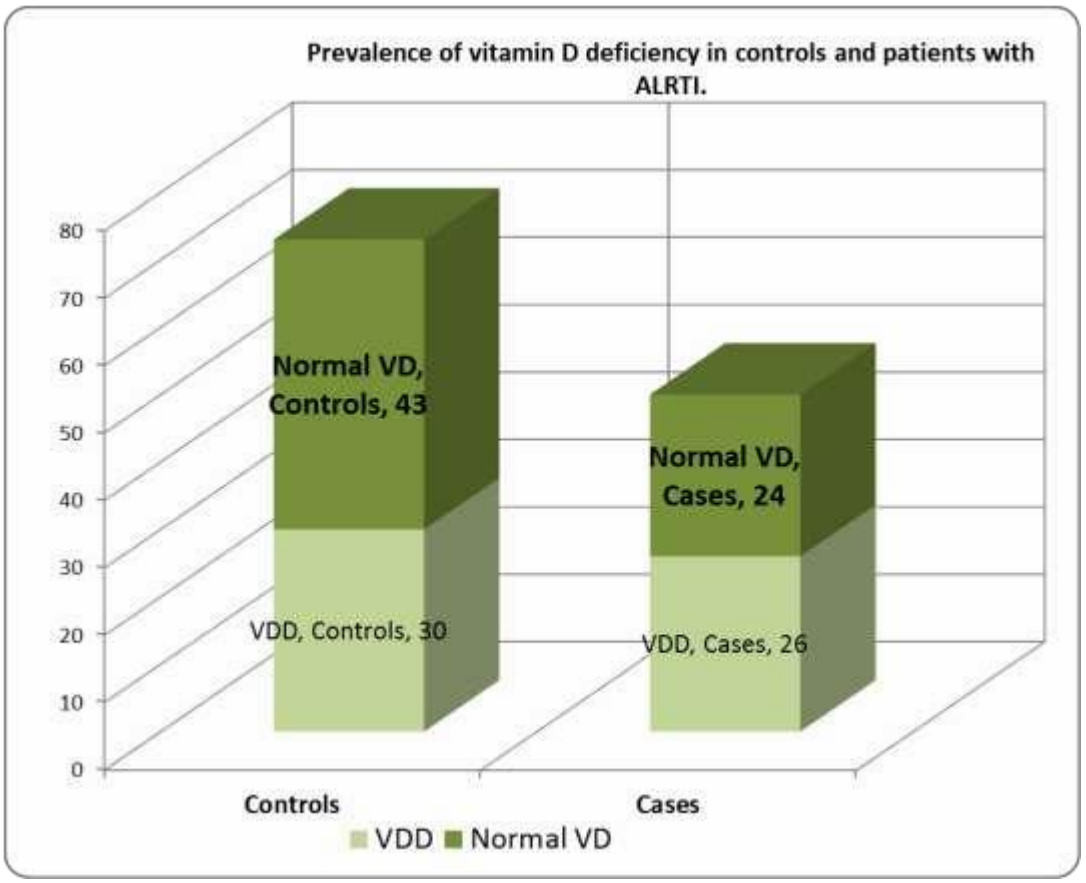
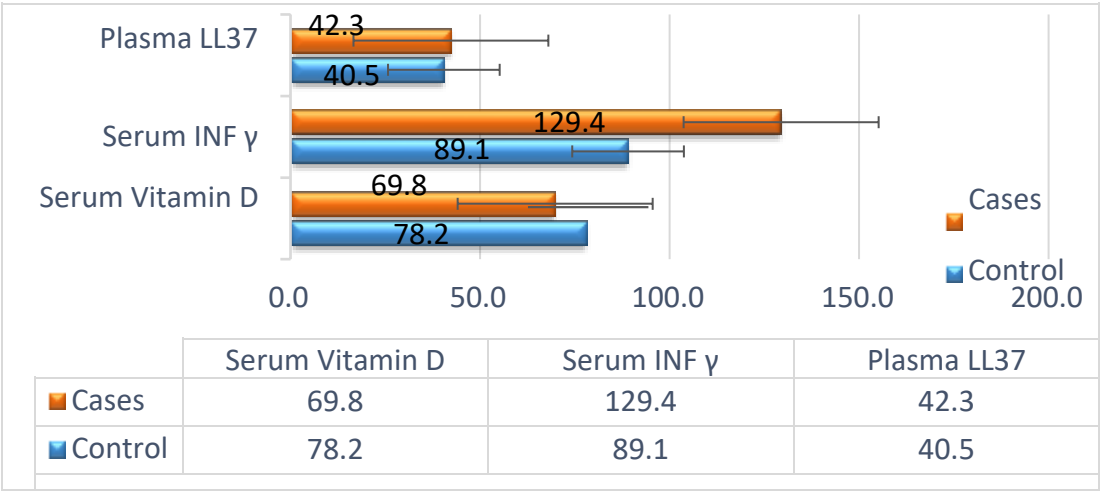


Figure 1. Prevalence of vitamin D deficiency in controls and patients with ALRTI



	Serum Vitamin D	Serum INF γ	Plasma LL37
Cases	69.8	129.4	42.3
Control	78.2	89.1	40.5

Figure 2. Serum vitamin D, LL37, and serum interferon-γ levels in controls and patients with ALRTI.

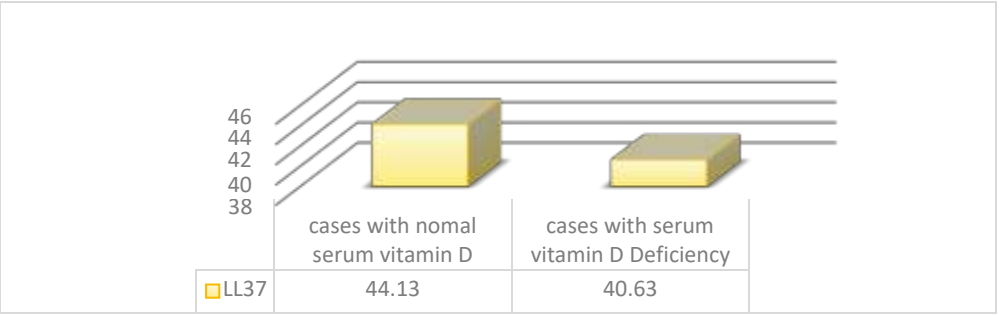


Figure 3. Plasma L37 in patients with ALRTI.

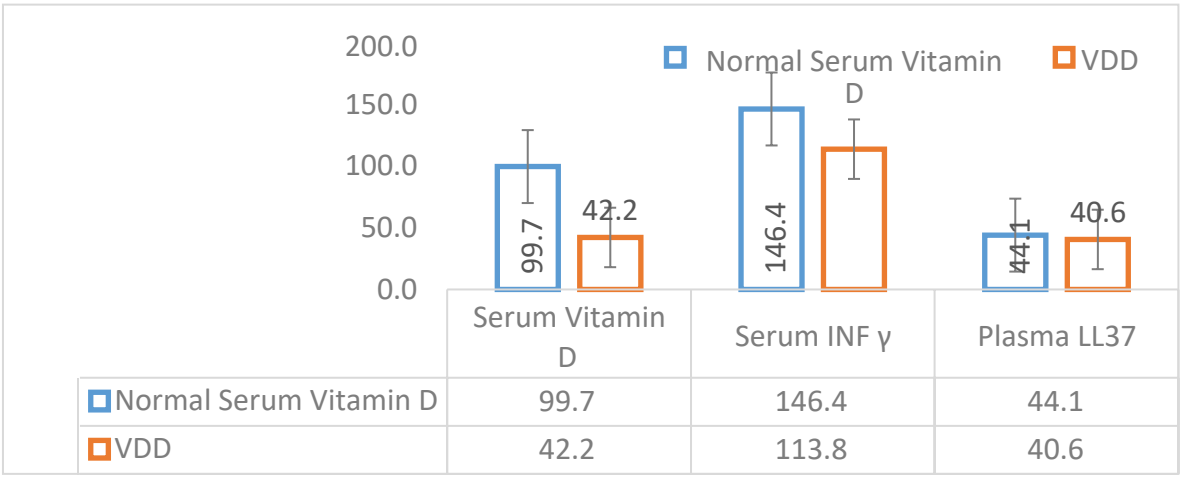


Figure 4. Serum vitamin D, serum interferon-γ, and plasma LL37 levels in patients with normal vitamin D or vitamin D deficiency.

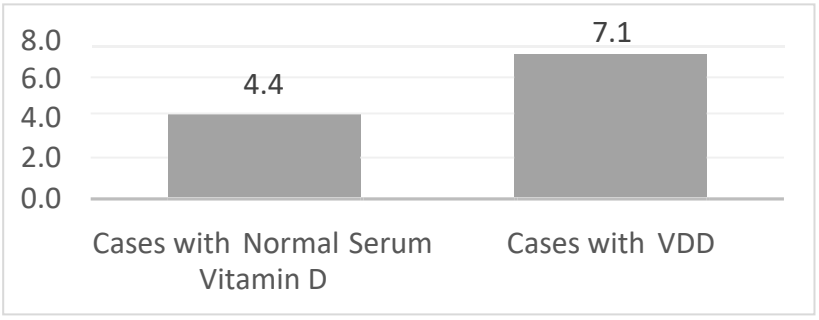


Figure 5. Lengths of hospital stay in patients with ALRTI according to vitamin D deficiency.

DISCUSSION

In this study, we evaluated the prevalence of serum vitamin D deficiency among Saudi children (1–10 years old) with or without ALRTI. Collectively, 46.3% of children included in this study had low serum vitamin D, i.e., serum vitamin D less than 75 mg/dl. For every 10 children who appeared healthy, there were four children with low serum vitamin D levels. This was increased to five of 10 children, i.e., about 50% of patients, when we investigated children with pneumonia or bronchopneumonia. Few studies have evaluated the prevalence of vitamin D among Saudi populations

in different geographical regions in Saudi Arabia. One study evaluated vitamin D levels in Dammam/KSA [7] and showed that there is a high prevalence of vitamin D deficiency in middle aged Saudi Arabians living in the Eastern region of the country. Another study targeted young women in Saudi schools in Jeddah [in the Western region] in 2004 and found that 81% of young girls in intermediate school had serum vitamin D deficiency [24]. Moreover, these findings are not restricted to KSA, but have also been observed worldwide. Reesukumal et al assessed the prevalence of vitamin D deficiency in apparently healthy children in Bangkok, Thailand [25] and found that despite exposure of children [6–10 years old] to sunlight, there was a high prevalence of vitamin D deficiency, i.e. 79.2% of subjects in that population. Similar results were found in Tehran, Iran [26], where 91.7 % of schoolchildren had serum vitamin D level less than 50 nM.

The current study showed that mean serum 25 vitamin D levels in patients with ALRTI were not significantly different from those in control, healthy children. Albanna et al also found no significant differences in serum vitamin D levels between patients with pneumonia and controls in an Egyptian population [21]. Moreover, similar results were observed in an Indian study [27]. In contrast, a Turkish study found that neonates with ALRTI had lower serum vitamin D levels than age-matched controls (22.8 versus 40.8 nM, respectively) [28]. Additionally, in rural areas in Bangladesh, mean serum vitamin D levels were significantly lower among patients with ALRTI than controls [29].

Data obtained from this study regarding the level of IFN-γ supported the role of this cytokine in inflammatory processes. Patients with ALRTI had significantly higher IFN-γ levels than children in the control group ($p < 0.001$). Increased vitamin D levels in patient serum can cause up regulation of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-2, IFN-γ, tumor necrosis factor-α, IL-6, IL-8, and IL-12, as well as type-1 T-helper cells and B-lymphocytes in the adaptive immune system [30]. Additionally, vitamin D is thought to have pleiotropic immunomodulatory effects and could be the major regulatory hormone of the immune system [31]. However, data obtained from this study regarding the level of IFN-γ showed that patients with ALRTI having low serum vitamin D levels did not have significantly different levels of IFN-γ compared with those in patients with normal vitamin D levels ($p < 0.31$).

Notably, the mean plasma LL37 level also did not significantly differ between patients with ALRTI having normal serum vitamin D or low serum vitamin D. However, it had showed that there was a positive relationship between serum vitamin D and LL37 [23]. Additionally, Albanna et al also showed that there is a positive correlation between serum vitamin D and plasma LL37 levels in patients versus controls [21].

Another prospective study conducted on 42 critically ill patients in the intensive care unit (ICU) at St. Vincent's Hospital in Sydney, Australia between January 2007 and January 2008 concluded that there was a high prevalence of hypovitaminosis D associated with adverse outcomes [8]. Additionally, in a recent French study conducted on 2010, researchers confirmed that vitamin D deficiency was a common finding in patients at the time of ICU admission [32].

Consistent with our current findings, [25] showed that there were no significant differences in vitamin D deficiency between patients with ALRTI and apparently normal controls; however, they showed that vitamin D deficiency may increase the severity of ALRT infection through its immunomodulatory properties [25]. Similarly, an American study performed on Caucasian Americans taking a daily dose of 2000 IU vitamin D3 revealed that the administration of vitamin D had no significant effect on the incidence or severity of ALRTI [33]. However, Inamo et al provided evidence supporting the immunomodulatory role for vitamin D, showing that there was a significant increase in the number of inpatients with ALRTI who received ventilation management in the group with low serum vitamin D levels [34].

Moreover, in a study of postoperative patients with severe and moderate vitamin D deficiency in the ICU, researchers showed that there was inverse association between serum vitamin D levels and length of stay [35]. Notably, in our study, we found that patients with ALRTI having low serum vitamin D levels had increased length of stay compared with that of patients with normal serum vitamin D levels. Overall, among 18 studies that investigated the correlations between serum vitamin D and risk of ALRTI, 13 studies [72.2%] showed a correlation between vitamin D deficiency and increased risk or severity of ALRTI [41].

Vitamin D plays an important role as a modulator of innate immunity [42]. The effects of vitamin D occur through different mechanisms. First, vitamin D affects T-helper cells [37]. Vitamin D stimulates Th-2 cells and subsequently

increases all cytokines secreted by these cells. Second, vitamin D regulates or stimulates Toll-like receptors [TLRs] [38]. Stimulated TLRs are responsible for recognition of bacterial lipopolysaccharide and viral proteins and subsequently enhances cytokine secretion.

Many clinical trials have evaluated the effects of vitamin D as a supplement in the course of ALRTI. Overall, the effects of vitamin D supplementation are better when the patient receives supplementation during the early phases of the infection. An Indian study performed in children reported improvement in treated cases [39]. Additionally, an American study performed in American Caucasians revealed that administration of vitamin D had no significant effect on acquisition of ALRTI with regard to either the incidence or severity of infection [33], similar to a British study performed in elderly patients [40].

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study revealed a high prevalence of vitamin D deficiency among Saudi children in general and among those with ALRTI. Low concentrations of vitamin D in patients with ALRTI were associated with increased length of hospital stay, potentially due to development of complications or treatment resistance.

No statistically significant differences in serum levels of vitamin D were observed between apparently healthy Saudi children and children with ALRTI. However, our findings provide insights into the potential application of vitamin D as a booster to reduce the risk of complications in patients with infection.

More studies are required to determine the prevalence of vitamin D deficiency among the Saudi population, with an emphasis on differences among age groups [particularly children] and clinical features. Although KSA is a sunny country during all seasons of the year, vitamin D deficiency is still a major health problem for various populations. Consequently, we recommend that vitamin D should be an essential part of the human diet or should be provided as a supplement to the diet.

REFERENCES

1. Hamada Y, Fukagawa M. The pleiotropic effects of vitamin D on kidney disease. *Clin Calcium*. **2007**; 17:712–7.

2. Holick MF. Vitamin D deficiency. *N Engl J Med*. **2007**; 357:266–81.

3. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *Am J Clin Nutr*. **2007**; 85[6]:6–18.

4. Suda T, Ueno Y, Fujii K, Shinki T. Vitamin D and bone. *J Cell Biochem*. **2002**;88:259–66

5. Hector F DeLuca. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr.* **2004**; 80:1689S–96S.

6. Raiten DJ, Picciano MF. Vitamin D and health in the 21st century: bone and beyond. *Am J Clin Nutr***. 2004**; 80:1673S–7S

7. Elsammak MY, Al-Wossaibi A, Al-Howeish A, Alsaeed J. High prevalence of vitamin D deficiency in the sunny Eastern region of Saudi Arabia: a hospital-based study. *East Mediterr Health J*. **2011**;17:317–22

8. Lee BP, Nair P, Eisman JA, Center JR. Vitamin D deficiency in the intensive care unit: an invisible accomplice to morbidity and mortality? *Intensive Care Med***. 2009**; 35:2028–32

9. Hancock, RE, Diamond G. The role of cationic antimicrobial peptides in innate host defenses. *Trends Microbiol***. 2000**; 8:402–10.

10. Bartley J. Vitamin D: emerging roles in infection and immunity. *Expert Rev Anti Infect Ther.* **2010**; 8:1359–69

11. Gudmundsson G H, Agerberth B. Neutrophil antibacterial peptides, multifunctional effector molecules in the mammalian immune system. *J Immunol Methods***. 1999**;232:45–54

12. Diamond G, Legarda D, Ryan LK. The innate immune response of the respiratory epithelium. *Immunol Rev.* **2000**; 173:27–38

13. Brogden KA. Antimicrobial peptides: pore inhibitors or metabolic inhibitors in bacteria? *Nat Rev Microbiol.* **2005**; 3:238–250

14. Gombart AF, Bhan I, Borregaard N, Tamez H, Camargo CA, Koeffler HP, Thadhani R. Low plasma level of cathelicidins antimicrobial peptide [hCAP18] predicts increased infectious disease mortality in patients undergoing hemodialysis. *Clin Infect Dis*. **2009;** 48 [4]:418–24.

15. Chennupati SK, Chiu AG, Tamashiro E et al. Effects of an LL-37-derived antimicrobial peptide in an animal model of biofilm Pseudomonas sinusitis. *Am J Rhinol Allergy*. **2009**;23:46–51.

16. Nizet V, Ohtake T, Lauth X et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature*. **2001**; 414:454–57

17. Yamshchikov AV, Desai NS, Blumberg HM, Ziegler TR, Tangpricha V. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. *Endocr Pract***. 2009**; 15:438-49

18. Liu PT, Stenger S, Li H et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science.* **2006;** 311:1770–3.

19. Muhe L, Lulseged S, Mason KE, Simoes E. Case–control study of the role of nutritional rickets in the risk of developing pneumonia in Ethiopian children. *Lancet*. **1997**; 349:1801–4

20. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D deficiency in young children with severe acute lower respiratory infection. *Pediatr Pulmonol*. **2009;** 44:981–8

21. Albanna EAM, Ali YF, Elkashnia RAM. Vitamin D and LL-37 in children with pneumonia. *Egypt J Pediatr Allergy Immunol***. 2010**; 8:81–86.

22. Gröndal G, Gunnarsson I , Rönnelid J , Rogberg S , Klareskog L , Lundberg I. Cytokine production, serum levels and disease activity in systemic lupus erythematosus. *Clin Exp Rheumatol*. **2000**;18:565–70

23. Merenstein D, Egleston B, Diener-West M. Lengths of stay and costs associated with children's hospitals. *Pediatrics*. **2005;** 115:839–44

24. Siddiqui AM, Kamfar HZ. Prevalence of vitamin D deficiency rickets in adolescent school girls in Western region, Saudi Arabia. *Saudi Med J***. 2007**; 28:441–4.

25. Reesukumal K, Manonukul K, Jirapongsananuruk O, Krobtrakulchai W, Hanyongyuth S, Chatsiricharoenkul S, Pratumvinit B. Hypovitaminosis D in healthy children in Central Thailand: prevalence and risk factors. *BMC Public Health*. **2015**; 15:248.

26. Neyestani TR, Hajifaraji M, Omidvar N et al. High prevalence of vitamin D deficiency in school-age children in Tehran, 2008: a red alert. *Public Health Nutr***. 2012**;15[2]:324–30.

27. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 years. *Eur J Clin Nutr*. **2004;** 58:563–7.

28. Ginde AA, Mansbach JM, Camargo CA Jr. Vitamin D, respiratory infections and asthma. *Curr Allergy Asthma Rep.* **2009**; 9:81–7.

29. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. *Acta Paediatr.* **2010;** 99:389–93

30. Baeke F, Takiishi T, Korf H, et al. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol*. **2010;** 10:482–96.

31. Baeke F, Gysemans C, Korf H, et al. Vitamin D insufficiency: implications for the immune system. *Pediatr Nephrol*. **2010**; 25:1597–606

32. Lucidarme O, Messai E, Mazzoni T, et al. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. *Intensive Care Med.* **2010**; 36:1609–11.

33. Li-Ng M, Alolia JF, Pollack S et al. A randomized control trial of vitamin D3 supplementation for the prevention of symptomatic upper respiratory tract infections. *Epidemiol Infect.* **2009;** 137:1396–404.

34. Inamo Y, Hasegawa M, Saito K et al. Serum vitamin D concentrations and associated severity of acute lower respiratory tract infections in Japanese hospitalized children. *Pediatr Int.* **2011**; 53[2]:199–201

35. Matthews LR, Ahmed Y, Wilson KL, Griggs DD, Danner OK, Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive care unit cost, and mortality rate in surgical intensive care unit patients. *Am J Surg***. 2012**; 204[1]:37–43.

36. Larkin A, Lassetter J. Vitamin D deficiency and acute lower respiratory infections in children younger than 5 years: identification and treatment. *J Pediatr Health Care*. **2014;** 28:572–82.

37. Beard JA, Bearden A, Striker R. Vitamin D and the anti-viral state. *J Clin Virol*. **2011;** 50:194–200

38. Oberg F, Botlin J, Nilsson K. Functional antagonisms between vitamin-D3 and retinoic acid in the regulation of CD14 and CD23 expression during monocytic differentiation of U-937 cells. *J Immunol.* **1993**; 150:3487–95

39. Rehman PK. Sub-clinical rickets and recurrent infection. *J Trop Pedatria*. **1994**; 40:58

40. Avenell A, Cook JA, Maclennan GS, Macpherson GC. Vitamin D supplementation to prevent infections: a sub-study of a randomized placebo-controlled trial in older people. *Age Ageing*. **2007;** 36:574–7.