VITAMIN D STATUS OF PEDIATRIC INTENSIVE CARE UNIT PATIENTS WITH PNUMONIA

By

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ABSTRACT

Introduction: *Vitamin D plays an important role, not only for bone health, but also for the immune system. Both in vitro and clinical studies have demonstrated that vitamin D is important for the innate and adaptive immune response. Adequate nutritional support has been a mainstay in pediatric intensive care unit (PICU) management with research showing improved outcomes and fewer PICU stay. However, there have been few studies to investigate the prevalence of vitamin D deficiency in critically ill children.*

Aim of the work: *This study aims to examine vitamin D status in the children with pneumonia who will be admitted to pediatric intensive care unit and correlate it to the severity and occurrence of complications.*

Patients and Methods: *This study was conducted in Pediatric intensive care unit at AlHussein University Hospital on 60 children; divided into two groups: group I (study group) including 40 patients presented with pneumonia. These patients aged from one month to fifteen years and admitted to the PICU of El-Hussein University Hospital (This group had been furtherly sub-classified to group Ia and group Ib according to occurrence of complications) and group II (control group) including 20 apparently healthy children of matched age and sex with no evidence of pneumonia or history of lower respiratory tract infection. Serum vitamin D was estimated.*

Results: *Statistically, the vitamin D level was significantly lower in study group than in control group, vitamin D level was significantly very low in cases with complications (group Ia) than in cases without complications (group Ib).There was a significant negative correlation between vitamin D level and occurrence of complications as well as duration of hospitalization.*

Conclusion: *This study concluded that vitamin D level was significantly lower in severe pneumonia which admitted to pediatric intensive care unit. There were correlation between vitamin D level and occurrence of complications and severity of pneumonia in*

children, and these patients with lower vitamin D level had longer duration of hospital stay.

Recommendations: *We recommend appropriate vitamin D supplementation and sun exposure to decrease the risk of respiratory tract infections, also recommend using vitamin D in addition to antibiotic as a treatment of pneumonia cases in children which has an important role in the production of anti-microbial peptides for innate immunity.*

Key words: *pneumonia, vitamin d deficiency, relation between vitamin D and pneumonia.*

INTRODUCTION

Pneumonia is an inflammatory condition of the lung especially affecting the microscopic air sacs (alveoli) associated with fever, chest symptoms, and a lack of air space (consolidation) on a chest X-ray **(Franco, 2017)**.

Pneumonia is the leading cause of death globally among children younger than age 5 years, accounting for an estimated 1.2 million (18% total) deaths annually. The incidence of pneumonia is more than 10-fold higher (0.29 episodes against 0.03 episodes), and the number of childhood-related deaths from pneumonia ≈2,000 fold higher, in developing than in developed countries. Fifteen countries account for more than threefourths of all pediatric deaths from pneumonia **(Matthew and Thomas, 2016)**.

Vitamin D (calciferol), which comprises a group of fat soluble vitamin that can be found in very few foods naturally, as well can be photosynthesized in the skin of vertebrates by the action of solar ultraviolet Beta radiation. In humans, vitamin D is unique because it can be ingested as cholecalciferol (vitamin D3) or ergocalciferol (vitamin D2) and because the body can also synthesize it (from cholesterol) when sun exposure is adequate (hence its nickname, the "sunshine vitamin") **(Saraff and Shaw, 2016)**.

Vitamin D has complex effects on pulmonary cell biology and immunity with impact on inflammation, host defense, wound healing, repair, and other processes. A number of epidemiological and experimental studies are available that highlight the relevance of this condition **(Saraff and Shaw, 2016)**.

Emerging evidence indicate that vitamin D mediated innate immunity, particularly through enhanced expression of the human cathelicidin antimicrobial peptide (hCAP-18) is important in host defenses against respiratory tract pathogens **(Kimlin et al., 2014)**.

A recent clinical investigation showed that high Vitamin D levels are associated with better lung function, less airway hyper responsiveness and improved glucocorticoid response **(Pfeffer and Hawrylowicz, 2018)**.

AIM OF THE STUDY

This study aims to examine vitamin D status in the children with pneumonia who will be admitted to our pediatric intensive care unit and correlate it to the severity and occurrence of complications.

ETHICAL CONSIDERATION

1. A written informed consent was obtained from all participants (parents) before participation in the study.
2. The objectives of the study, the expected benefits and types of information to be obtained were explained to them.
3. An approval by the local ethical committee was obtained before the study.
4. The author declared no potential conflicts of interest with respect to the research, authorship and/or publication of the article.
5. All the data and results of the study are confidential and the participants had right to keep it.
6. The patient has the right to withdraw from the study at any time.

MATERIALS AND METHODS

All patients were collected from Pediatric ICU department at Al-Hussein University Hospital over a period from November 2015 to December 2016, with written parental consent to participate in the study.

Patients:

All children in the study are sequentially enrolled according to the study design and the inclusion criteria, they were classified into two groups as follow:

Group I (study group):

Comprising 40 patients presented with pneumonia. These patients aged from one month to

fifteen years and admitted to the PICU of El-Hussein University Hospital at the time zone of the study, this group had been furtherly sub-classified to group Ia and group Ib according to occurrence of complications.

Group Ia: which represented patients with pneumonia and complications e.g. (pneumothorax, pleural effusion, empyema, respiratory failure, septic shock….etc) they were 14 child.

Group Ib: which represented patients with uncomplicated pneumonia they were 26 child.

Group II (control group):

Comprising 20 apparently healthy children of matched age and sex with no evidence of pneumonia or history of lower respiratory tract infection.

Inclusion criteria:

All patients in the group I must fulfill the following criteria: age from 1 month to 15 years, Presented by signs and symptoms of pneumonia (cough, fever, dyspnea,…etc) confirmed by positive radiological and/or culture results and admitted to the pediatric ICU department.

Exclusion criteria:

Any child with any disorder can affect vitamin D absorption or metabolism (e.g. mal-absorption syndrome, hepatic or renal impairment), child with immune deficiency syndromes (diagnosed or suspected), failure to thrive or any nutritional deficiency syndromes had been excluded from the study.

Methods:

All patients were subjected to the following:

I) Full history taking including: - personal history, complaint, present history, Developmental history, Dietetic history, vaccination history, past history, perinatal history and family history.

II) Medical examination including:- general, local and Systemic Examination.

III) Routine investigations e.g. CBC, blood gases, radiographs…….etc.

IV) Vitamin D status will be assessed for all children in both groups by measurement of serum 25-Hydroxy vitamin D (25 (OH) D). Currently,

Vitamin D status is categorized based on Endocrine society guidelines as deficiency, insufficiency, and sufficiency based on serum 25-OH Vitamin D levels below 20 ng/ml (50 nmol/L), 21–29 ng/ml (52.5– 72.5 nmol/L), and 30–100 ng/ml (75–250 nmol/L), respectively. Although this classification is accepted widely, extensive efforts are being taken to interpret the basis of this criteria as most of the global populations are found to be Vitamin D deficient **(Selvarajan et al., 2017)**.

V) Sampling; Eight ml venous blood sample were withdrawn from all participants of the study (both patients and controls) and divided into two portions: the first portion (two ml) was put in EDTA tube for CBC. The second portion (six ml) was put in plain tube and left to clot for 30 minutes then serum was separated and divided into two aliquots, one for routine biochemical tests performed on same day of collection by an enzymatic colorimetric reaction using a Modular P analyzer (Roche Diagnostics) while the other aliquot was stored deeply frozen at - 800 for estimation of serum 25(OH) D3 using ELISA.

Determination of 25hydroxyvitamin D [25(OH) D]: using a commercially available (ELISA) kit supplied by ORGENTEC Diagnostika GmbH, Germany.

Principle:

The determination is based on a competitive enzyme linked immune-sorbent assay (ELISA), Antibodies detecting 25-OH Vitamin D2 and 25-OH Vitamin D3 are bound onto micro-wells.

In a first step 25-OH vitamin D contained in the sample has to be released from its vitamin D binding protein: The undiluted sample is placed in a test tube and mixed with sample buffer containing 25-OH vitamin D tracer reagent and then with vitamin D release reagent. The released 25-OH vitamin D sample is transferred to reaction wells of the microtiter plate. 25-OH vitamin D in the sample competes with the 25-OH vitamin D tracer reagent for binding to the 25-OH vitamin D

antibodies coated onto the microwells. Complexes are formed between antibody and 25OH vitamin D or antibody and 25OH vitamin D tracer. After incubation, a first washing step removes unbound and unspecifically bound molecules. Subsequently added enzyme conjugate binds to the immobilized tracer-antibody complexes. After incubation, a second washing step removes unbound enzyme conjugate. Addition of enzyme substrate solution results in blue color development during incubation. Addition of an acid stops the reaction generating a yellow endproduct. The intensity of the yellow color correlates inversely with the concentration of vitamin D in the sample and can be measured photo-metrically at 450 nm.

Test Procedure:

1. Pipette 100 µl of pretreated patient samples, calibrators and controls into the wells. Incubate for 30 minutes at room temperature (20-28 °C).Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
2. Dispense 100 µl of enzyme conjugate into each well. Incubate for 15 minutes at room temperature. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
3. Dispense 100 µl of TMB substrate solution into each well. Incubate for 15 minutes at room temperature.
4. Add 100 µl of stop solution to each well of the modules. Incubate for 5 minutes at room temperature.

Using Tecan spectra (ELISA reader) read the optical density at 450 nm (reference 600-690nm) and calculates the results. The developed colour is stable for at least 30 minutes. Read during this time.

RESULTS

In our study, group I comprises 40 patients (24 males

and 16 females), while group II comprises 20 apparently healthy child (12 males and 8 females). As regard age distribution of group I (12.93 ± 1.118) month meanwhile it was (13.85 ± 2.124) month for group II and there was no statistically significant difference between both groups regarding age or sex distribution.

Regarding the laboratory findings of both groups there were statistically significant difference between group I and group II in white blood cells count, hemoglobin concentration, ESR, CRP (P value <0.001) while there was no statistically significant difference between them in platelets count (P value >0.05).

There was highly significant difference between both groups regarding the vitamin D level. Vitamin D level of both groups is shown in (table I).

Table (I): Vitamin D Level of both Groups:

	Group I (N=40) Mean ± SD	**Group II (N=20) Mean ± SD**	**P value**
Vitamin D level (ng/ml)	11.42 ± 0.6532	44.20 ± 2.961	0.001
Number of Patients with Sufficient Levels (30–100 ng/ml)	0	13	0.001
Number of Patients with Insufficient Levels (21–29 ng/ml)	11	3	0.001
Number of Patients with Deficient Levels (below 20 ng/ml)	29	4	0.001

P<0.001: highly significant, P>0.05:non-significant

Out of 40 patients in group I, 14 had complications during the course of the disease which comprises the group Ia. The distribution of these complications as follow: respiratory failure (6 cases), pleural effusion (3 cases), empyema (1 case), pneumothorax (2 cases) and sepsis (2 cases).

Table (II): Comparison between Cases with Complications (Group Ia) and Cases without Complications (Group Ib):

	Group(Ia) (N=14) Mean ±SD	Group (Ib) (N=26) Mean ±SD	P value
Age (month)	6.5±7.1	12.6±7.5	0.4
Temperature (°C)	39.4±0.4	37.9±0.2	0.001
Respiratory Rate (cycle/minute)	72.5±4.3	63.2±4.2	0.001
WBC (×1000 cells/µl)	18.03 ± 3.5	12.5 ± 0.8	0.001
Hb (gm/dL)	10.6 ± 0.98	12.1 ± 1.04	0.0197
Platelets (×1000 platelets/µl)	206 ± 68.6	280.8 ± 51.6	0.3
ESR	39.9 ± 8.1	22.1 ± 2.5	0.001
CRP **+ve** **-ve**	 14 0	 26 0	0.023
Vitamin D level (ng/ml)	6.9 ± 9.8	13.1 ± 30.96	0.001

P<0.001: highly significant, P>0.05:non-significant

White blood cells count, hemoglobin concentration, ESR and vitamin D level were significantly different in group Ia (cases with complications) and group Ib (cases without complications) (P value <0.001) as shown in (table II).

The correlation between the vitamin D level and occurrence of complications was highly significant negative correlation (R=-0.21, P value <0.001) as shown in (fig. I).

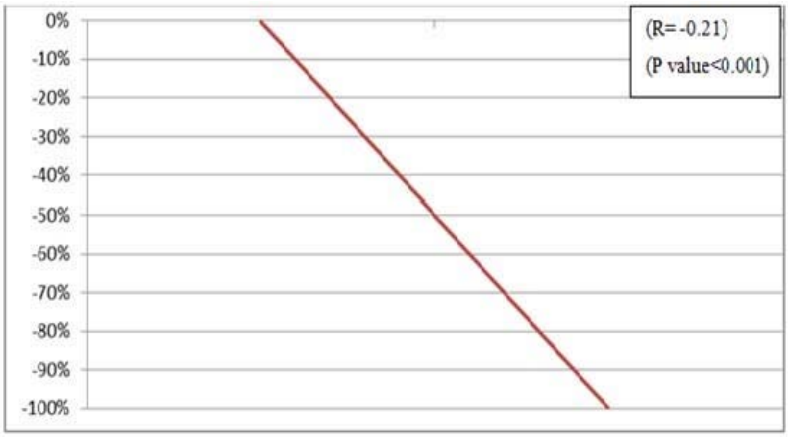

Figure (I): Correlation between vitamin D level and occurrence of complications

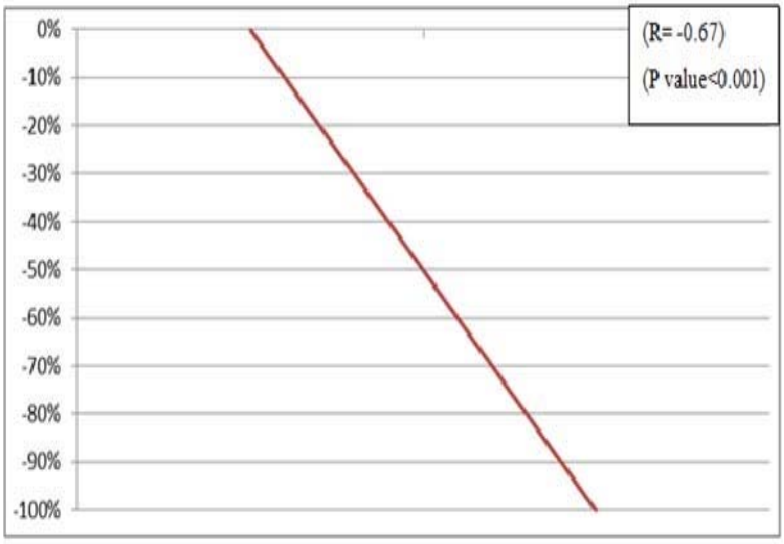

Figure (II): Correlation between Vitamin D Level and Hospital Stay.

Mean duration of admission for all cases (group I) was (7.5 ± 8.5) days with highly significant negative correlation between the duration of hospital stay and vitamin D level (R=-0.67, P value <0.001). This correlation is shown in (fig II).

DISCUSSION

We conducted our study on 60 candidates 40 as case group (group I) have pneumonia and admitted to the pediatric intensive care unit, while the other candidates comprises the control group (group II) and we found that: patients with pneumonia (study group) had a higher rate of severe vitamin D deficiency in comparison with healthy participants (control group) (P value <0.001), furthermore, patients with severe vitamin D deficiency (study group) had more severe disease (occurrence of complications), and there was statistically significance between patients with severe deficiency and a higher rate of longer duration of hospital stay.

So our study in agreement with the study done by **(Mojgan et al., 2017)** According to findings, a low level of 25(OH) D is associated with a higher incidence of community-acquired pneumonia and more severe disease .

As well as the study done by **(Özdemir et al., 2016)** found significantly low serum 25 (OH) D levels in children with recurrent respiratory infections (RRI).Their study conducted on 98 child with recurrent respiratory tract infections and 124 healthy children as a control group.

Comparable to our results in a study done by **(Kim et al., 2015)** show the prevalence of vitamin D deficiency was ~80% in patients hospitalized with communityacquired pneumonia.

(Aregbesola et al., 2013) investigated the effect of 25(OH) D on the risk of incident hospitalized pneumonia in an aging general population in eastern Finland. They found that the subjects with the lowest level of serum 25(OH) D had a significant 2.6-fold (95% CI: 1.4, 5.0) higher risk of developing pneumonia compared to the subjects with the highest levels. They concluded that there was an inverse effect of serum 25(OH) D concentration on the risk of incident pneumonia in the aging population.

(Quraishi et al., 2013) reported the result of the third National Health and Nutrition Examination Survey in a large nationally representative cohort of

non-institutionalized adults in the United States. They showed that after adjusting for demographic factors, clinical data, and season, 25 (OH) D levels less than 30 ng/mL were associated with 56% higher risk for pneumonia.

In the same stream another study done by **(Lin-Ying et al., 2017)** they said that vitamin D is involved in regulating innate and adaptive immune functions; we found that low vitamin D status is related to the susceptibility to Viral pneumonia in children, and the degree of deficiency affects the critical conditions of Viral pneumonia cases. To understand whether vitamin D supplementation is beneficial for preventing and treating viral pneumonia in children, further large-sample, trans-regional, polycentric studies would clarify its role in children.

As well as the study done **(Pletz et al., 2014)** said that for 1, 25(OH) 2 vitamin d, we found a significant and independent (controlled for age, season and pathogen) negative correlation to pneumonia severity. Therefore, supplementation of non-activated vitamin D to protect from pneumonia may be non-sufficient in patients that have a decreased capacity to hydroxylate 25-OH to 1, 25 (OH) 2.

Contrary to our study **(Yakoob et al., 2016)** who said that Evidence from one large trial did not demonstrate benefit of vitamin D supplementation on the incidence of pneumonia or diarrhea in children less than five years. To our knowledge, trials that evaluated supplementation for preventing other infections, (including TB and malaria) have not been performed. Another study done by **(Roth et al., 2009)** who did not reveal an association between vitamin D deficiency and respiratory tract infections in three smaller case control studies and attributed this to that 95% 0f the participants received some forms of vitamin D supplementation, thereby increasing the serum 25(OH) D in the placebo group.

(Larkin and Lassetter, 2014) they found that Of 18 studies examined, vitamin d deficiency was found to be associated with increased risk or severity of acute lower respiratory infection in 13 studies; associations were not found in 4 studies. In one study it

was found that high maternal vitamin D levels were associated with acute lower respiratory infection in infants.

In a study done by **(Martineau et al., 2017)** evaluating the Vitamin D supplementation to prevent acute respiratory tract infections said that Vitamin D supplementation was safe and it protected against acute respiratory tract infection overall. Patients who were very vitamin D deficient and those not receiving bolus doses experienced the most benefit.

We not the only found a significant association between vitamin D levels and occurrence of complications in pneumonia, also some studies indicated a significant relationship; for instance, **(Remmelts et al., 2012)** in a prospective cohort study on hospitalized patients with CAP stated that vitamin D deficiency was associated with an increased risk of ICU admission and 30-day mortality. Also, they showed that 25(OH) D status was an independent predictor of 30-day mortality and concluded that there was a relationship between deficiency and adverse outcome in CAP .

(Amrein et al., 2014) said that among critically ill patients with vitamin D deficiency, administration of high-dose vitamin D3 compared with placebo did not reduce hospital length of stay, hospital mortality, or 6-month mortality. Lower hospital mortality was observed in the severe vitamin D deficiency subgroup, but this finding should be considered hypothesis generating and requires further study.

However, **(Choudhary and Gupta., 2012)** evaluated the role of oral vitamin D supplementation (1000–2000 IU per day for 5 days) for resolution of severe pneumonia in a randomized controlled trial on 200 children under 5 years of age. They found that median duration for the resolution of severe pneumonia was similar in both the groups (vitamin D supplementation vs placebo). Also, the two groups did not differ in duration of hospitalization and time to resolution of tachypnea, chest retractions, and inability to feed. They stated that short-term

supplementation with oral vitamin D has no beneficial effect on resolution of severe pneumonia in under-five children. The fact that the short-term supplement of vitamin D cannot produce results in patients with CAP can be due to the long-term effects of vitamin D on the maturation and development of lymphocytes and other mononuclear cells in the immune system.

We also found negative correlation between occurrence of complications among the cases of pneumonia and their vitamin D level, also the same statistically significant negative correlation between the vitamin D level and the duration of hospital stay (R= 0.67, P value <0.001) but we can't attain other studies concentrate on these points for comparison of results and discussion.

CONCLUSION

This study concluded that vitamin D level was significantly lower in sever pneumonia need admission to pediatric intensive care unit, there were correlation between Vitamin D level and occurrence of complications and severity of pneumonia in children, and these patients with lower vitamin D level had longer duration of hospital stay.

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الملخص العربي

أجري ت ه ذه الدراس ة بالتع اون ب ين أقس ام عل م الأم راض الس ريري وطب الأطفال في مستشفى الحسين الجامعي، كلية الطب، جامعة الأزهر.

ت م جم ع جمي ع المرض ى م ن قس م ط ب الأطف ال بمستش فى الحس ين الج امعي خ لال الفت رة م ن ن وفمبر 2015 إل ى ديس مبر 2016، م ع أخ ذ الموافق ة المناس بة عل ى المش اركة ف ي ه ذه الدراس ة م ن أه ل المرض ى بع د ش رح طبيع ة الدراس ة وك م ه و مفي د في التشخيص والعلاج و أنه مجرد جمع عينات الدم.

أ- المجموعات:

المجموعة الأولى (مجموعة المرضى:)

ش ملت 40 مريض اَ م ع أع راض وعلام ات الإته اب الرئوي (أع راض وعلام ات الالتهاب الرئوي بالص در واش عة الصدر أو مزرع ة ال بلغم) ال ذين تت راوح أعم ارهم ب ين ش هر واح د وخم س عش رة عام ا، والمحج وزين ف ي وح دة العناي ة المرك زة للأطف ال ف ي مستش فى الحس ين الج امعي والت ي ش ملت 24 م ن ال ذكور و 16 م ن الإناث.ه ذه المجموع ه انقس مت ال ي مجم وعتين : المجموعه الاول ي (أ) (مجموع ة المرض ي ال ذين ح دثت له م مض اعفات) و المجموع ه الاولي (ب) (مجموعة المرضي الذين لم تحدث لهم مضاعفات.)

المجموعة الثانية (المجموعة الضابطه:)

تض م 20 م ن الاطف ال الأص حاء م ع ع دم وج ود دلي ل عل ى الالته اب الرئوي أو تاريخ عدوى الجهاز التنفسي.

معايير الاشتمال:

ش ملت الدراس ه الرض ع والأطف ال م ن عم ر ش هر واح د إل ى خمس ة عش ر عاما وال ذين طلب وا الحص ول عل ى المش ورة الطبي ة ف ي العي اده الخارجي ه للأطف ال و ت م حج زهم ف ي قس م العناي ة المرك زة للأطف ال ف ي مستش فى الحس ين م ع أدل ة عل ى الالته اب الرئ وي (أع راض وعلام ات الالته اب الرئ وي بالص در وأدل ة الأش عة أو فح ص مزرع ة ال بلغم) بغ ض النظ ر ع ن مس ببات أو ش دة المرض.

وقد استند تشخيص الحالات على الآتى:

- ت اريخ وأع راض ع دوى الجه از التنفس ي الح ادة (بم ا ف ي ذل ك الس عال والحم ى وضيق التنفس .. الخ).

- المظ اهر الس ريرية م ع وج ود أدل ة عل ى الالته اب الرئ وي (أع راض وعلام ات الالته اب الرئ وي بالص در والأش عة أو فح ص مزرع ة ال بلغم) بغ ض النظ ر عن مسببات أوشدة المرض.

معايير الاستبعاد:

جميع الرضع أقل من شهر واحد من العمر.

ب- طريقة العمل:

تم خضوع جميع المرضى لما يلي:

1- تم أخذ التاريخ المرضي الكامل.

2- الفحص الطبي البدني الشامل.

3- التحالي ل الروتيني ة عل ى س بيل المث ال: ص ورة دم كامل ه ، ب روتين س ي النش ط ، سرعة الترسيب ، أشعة عادية على الصدر (للمجموعه الاولي فقط.)

4- تم قياس نسبة فيتامين (د) لجميع المرضى.

نتائج البحث:

ولق د أثبت ت الدراس ة ان ع دد ك رات ال دم البيض اء وس رعة الترس يب أعل ي وذو دلال ه أحص ائيه ف ي المجموع ة الأول ى (المرض ي) ع ن المجموع ه الثاني ه (الأص حاء) بينم ا مس توى الهيموجل وبين أق ل وذو دلال ه أحص ائيه ف ي المجموعه الأولي (المرضي) عن المجموعه الثانيه (الأصحاء.)

ووج د أن مس توي فيت امين د أق ل وذو دلال ه
أحص ائيه ف ي المجموع ه الأولي (المرضي) عن المجموعه الثانيه (الأصحاء.)

ووج د أن مس توي فيت امين د أق ل وذو دلال ه
أحص ائيه ف ي المجموع ه الأول ي (أ) (المرض ي ال ذين ح دثت له م مض اعفات) ع ن المجموع ه الأول ى
(ب) (المرضي الذين لم تحدث لهم مضاعفات.)

وق د أثبت ت الدراس ه أيض ا ان هن اك علاق ه عكس ية ب ين مس توي فيت امين د ف ي ال دم ونس بة ح دوث مض اعفات للطف ل الم ريض حي ث ل وحظ ح دوث مضاعفات شديده في الاطفال الذين كان مستوي فيتامين د في دمهم قليل جدا.

ووج د ايض ا ان هن اك علاق ه ب ين ال نقص الش ديد لمس توي فيت امين د ف ي ال دم و م دة حج ز الطف ل المص اب بالالته اب الرئ وي ف ي المستش في كلم ا ق ل مس توي فيت امين د ف ي ال دم كلم ا ت اخر ش فاء الطف ل وكلم ا ط ال بقائ ه ف ي المستشفي للاستشفاء .

وعل ى ض وء ه ذه النت ائج يمكنن ا الاس تنتاج أن ه عن د أنخف اض مس توى

فيت امين د يترت ب علي ه أنخف اض ق درة الجه از المن اعي للجس م عل ى مقاوم ة ع دوى الجه از التنفس ي ع ن طري ق قل ة اف راز المض ادات الحيوي ه الطبيعي ه الت ي تعتم د عل ى فيت امين د وبالت الي زي ادة نس بة ح دوث وخط ورة الإلتهاب الرئ وي عند الأطفال.