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Research Article

Frequency of 25-Hydroxyvitamin D Deficiency in Pediatric Patients with Immune Thrombocytopenia: Disease Phase and Therapy Options

Huda K. Abbas[1], Basil O. Saleh*[1], Hasanein H. Ghali[2]
[1] *Department of Clinical Biochemistry, College of Medicine, University of Baghdad, Baghdad, Iraq.*
[2] *Department of Pediatric, College of Medicine, University of Baghdad, Baghdad, Iraq.*

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Abstract

Background: Hypovitaminosis D can cause immunological irregularities in the development of immune thrombocytopenia.

Objectives: To identify the frequency of low levels of 25-hydroxyvitamin D in children with Immune thrombocytopenia (ITP), and to assess the effect of the disease phase and type of treatment on vitamin D level.

Methods: This case-control study was carried out on 88 children (63 had been diagnosed with immune thrombocytopenia and 25 healthy children as controls) during November 2023 and April 2024. The patients were sub-grouped according to global classification of vitamin D level into three groups: less than 10 ng/ml (n = 47), 10–20 ng/ml (n = 16), and 20-30 ng/ml (none of the patients or controls fell in this group). The cases were sub-classified according to their disease phase: Acute (n = 21), persistent (n = 24), and chronic (n = 18)**.** The serum 25-hydroxyvitamin D level was measured using the enzyme-linked immunosorbent assay (ELISA) technique.

Results: Around 75% of ITP children had a serum 25-hydroxyvitamin D level of less than 10 ng/ml. The mean (± SEM) values of the serum 25-hydroxyvitamin D of the ITP children of acute (9.5±1.84 ng/ml) and chronic (8.0±1.13 ng/ml) phases were lower than those of controls (10.0±1.32 ng/ml, $p > 0.05$), but not significantly so. The mean values of 25 hydroxyvitamin D of ITP children were lower than those of the controls, irrespective of the type of treatment.

Conclusion: Vitamin D deficiency is prevalent among children with immune thrombocytopenia, particularly those in the chronic phase.

Keywords: Autoimmune; Immune Thrombocytopenic; Purpura; Thrombocytopenic; 25-hydroxy vitamin D.

Introduction:

Immune thrombocytopenia (ITP) is an autoimmune disorder defined by the immune system's destruction of platelets in the peripheral blood. The imbalance between the rates of platelet generation and elimination in the bone marrow leads to different levels of vulnerability to bleeding. A minority of patients experience severe hemorrhaging that poses a risk to their lives. ITP is diagnosed when the platelet count falls below 100,000 per cubic millimeters (1). Most patients present with skin bleeding including purpura and skin ecchymoses, and nasal or oral bleeding. Increased menstrual bleeding and urogenital hemorrhage were reported (2). ITP is classified as newly diagnosed, persistent, or chronic, based on the duration of the disease. Newly diagnosed ITP is defined as a disease diagnosed within three months of the onset of thrombocytopenia symptoms. Persistent ITP is the disease that lasts 3-12 months. A chronic form that lasts more than 12 months (3). ITP is the most prevalent cause of acquired thrombocytopenia in childhood, affecting 2 to 5 / 100 000 Children (4). Vitamin D insufficiency is prevalent in both developed and developing countries. In the United States, it has an equal impact on children and adults (5). Studies have demonstrated that vitamin D has a considerable impact on both innate and adaptive immune responses. It can improve phagocytosis and control the activity of the T helper and regulatory cells (6–8). Immunological abnormalities leading to chronic ITP can be caused by hypovitaminosis D, and treating with vitamin D can alter the immune system and lower the likelihood of chronic disease (9). Children with ITP (whether newly diagnosed, persistent, or chronic) typically have vitamin D insufficiency. Vitamin D has been suggested as a potential treatment for autoimmune illnesses due to the correlation between vitamin D and the occurrence or severity of these diseases(10). Many children with ITP, similar to those with other autoimmune disorders, frequently suffer from hypovitaminosis D. Children with hypovitaminosis D have more severe ITP, suggesting that vitamin D therapy could be a

*Corresponding Author: basil_omsal@comed.uobaghdad.edu.iq,

novel strategy to treat this disease (11). Patients with autoimmune diseases have a higher prevalence of vitamin D insufficiency compared to healthy individuals. Glucocorticoids, a type of drug used to treat autoimmune illnesses, are a risk factor for vitamin D insufficiency (12). The increased prevalence of hypovitaminosis D in autoimmune illnesses is not entirely understood. Excessive use of corticosteroids may partly explain this phenomenon. It leads to increased breakdown of vitamin D by increasing the expression of certain receptors and enzymes, such as steroids and xenobiotic nuclear receptors (SXR) and CYP3A4, respectively (13). The study conducted in Egypt on primary ITP patients who were less than 18 years old, both sexes, found that the Vitamin D values are significantly lower in chronic and persistent ITP children than those in controls (6). Another study conducted in Croatia on 2–18-year-old children found that Vitamin D deficiency is very common in children with both newly diagnosed and chronic ITP forms. Innate and adaptive immune responses are modulated by vitamin D, an immunomodulatory drug that targets a variety of immune cells, including monocytes, macrophages, dendritic cells (DCs), T lymphocytes, and B lymphocytes. Vitamin D also decreases the likelihood of developing autoimmune illnesses. Furthermore, there are indications that autoimmune illnesses are susceptible to vitamin D. Significant amounts of Vitamin D receptors (VDR) are present in T- lymphocyte and macrophage populations, and they play an important role in T cell-mediated immunity (14).

Increased platelet count, less bleeding, remission induction, and overall patient well-being are the goals of immune thrombocytopenia treatment. Corticosteroids serve as the first line of defense against platelet destruction by preventing the development of autoantibodies and excessive cytotoxic T-cell activity. Other possible treatment options include Intravenous immunoglobulin, mycophenolate, and thrombopoietin receptor agonists (TPO-RAs) in the event of bleeding affecting lifestyle, as well as platelets transfusion in cases of life-threatening bleeding in rare cases (15). Thus, the aim of the current study was to determine how often children with ITP have low levels of 25-hydroxyvitamin D and to evaluate how the disease stage and therapy type affect vitamin D levels.

Patients and Methods:

This study was conducted in the Children Welfare Teaching Hospital, Medical City, Baghdad, Iraq, by the Department of Biochemistry / College of Medicine / University of Baghdad, between November 2023 and April 2024. The study included 88 children, 63 of them had been diagnosed with ITP by a pediatric hematologist, and 25 healthy children as controls who were free from any acute and chronic illnesses, they were selected from the children of colleagues and relatives from Baghdad and other governments after details asking about their medical history. The ITP group and control group were sub-classified according to their serum vitamin D levels into three groups: Those with less than 10 ng/ml of vitamin D, those with vitamin D levels from 10 to 20 ng/ml, and those whose vitamin D levels ranged from 20 to 30 ng/ml (16).

The ITP children were also sub-classified according to the disease phase into The ‘newly diagnosed ITP group’ (ITP duration within three months), the ‘persistent ITP group’ (ITP duration between 3 - 12 months), and the ‘chronic ITP group’ (ITP duration that is more than 12 months) (3). The ITP children included in this study were also sub-classified according to their type of treatment into Group 1 (Romiplostim therapy group), which included 18 children, Group 2 (the steroid therapy group), which included 19 children treated with prednisolone only, and Group 3 (other modality including; prednisolone and IVIG, or prednisolone and mycophenolate) included 26 children treated with prednisolone and IVIG, or prednisolone and mycophenolate. The dose of each medicine was defined by a consultant pediatric hematologist according to the severity of the disease. It has to be mentioned that the platelets count reported in the study was the count at the time of the patient’s visit to the outpatient clinic in the hospital. Some of these counts are normal as a response to treatment while others were still low during treatment.

The cases were selected at the age range of 1 - 16 years, and they were all undergoing treatment. The first line of their treatment was prednisolone alone or in combination with intravascular immunoglobulin (IVIG). The second line was mycophenolate. The third line was Romiplostim.

This study was approved by the scientific and ethical committees of the Department of Biochemistry, College of Medicine, University of Baghdad. Ethical approval was also obtained from the Children Welfare Teaching Hospital, Medical City, and Ministry of Health / Iraq. Verbal consent was obtained from the children's guardians in this study.

The control group consisted of 25 healthy children selected from the children of colleagues and relatives who were healthy and not suffering from any acute or chronic illness.

The exclusion criteria included those patients who had a blood transfusion during the previous month, had active infections, and any case of suspected inherited platelet disorders based on history, physical examination, and laboratory results.

Five milliliters (ml) of blood was aspirated from the peripheral vein of each patient and control group and allowed to clot for 15 minutes, then centrifuged for 10 minutes at 2500 rpm. The separated serum was stored at -45º C till the day of lab testing, which included measurements of vitamin D, using a semiautomatic ELISA Reader (Huma Reader, by the Human Diagnostics German company, Washer (COMBIWASH)). The principle of the ELISA technique with the biotin double antibody sandwich method was used for the evaluation of human vitamin

D. The wells were coated with the vitamin D monoclonal antibody and allowed to incubate. The next step was to combine streptavidin-HRP with biotin-labeled anti-vitamin D antibodies, to create an immunological complex. After incubation and washing, the enzymes that remained unbound were removed. Substrates A and B were combined. In the presence of acid, the solution would undergo a color shift, first from blue to yellow. The human vitamin D content was positively correlated with the solution color. The platelet counts were measured using Huma Count 30 ^{TS} Human, Germany.

Statistical analysis was done using the SPSS version 25.0 software which described the data using percentages, means, and standard error of the mean (SEM). The ANOVA test was used to assess the differences between means of numerical data when more than two means were tested. The correlation between the numerical data was evaluated using the Pearson correlation regression. A P value of < 0.05 was considered significant.

Results:

Out of the 63 ITP children, there were 34 females (54%) and 29 males (46%). Table 1 shows the mean (±SEM) values of age, platelet counts, and 25-hydroxy vitamin D in the blood of ITP children and controls. The mean platelet count value was significantly lower in the ITP children compared to the controls (p = 0.0001). ITP children's serum 25-hydroxy vitamin D mean value was very low but not significantly different from the controls.

Table 1: Mean (± SEM) of age, platelet counts, and 25-hydroxy vitamin D in the ITP cases and controls

Parameter	ITP group (n=63)	Controls (n=25)	*P* Value
Age (years)	7.2±0.51	8.3±0.93	> 0.05
Platelet count (10^9/L)	117.5±18.15*	376.2±7.51	< 0.0001
25-hydroxy vitamin D (ng/ml)	7.9±0.50	10.0±1.32	> 0.05

Table 2 shows the percentage of cases and controls in each of the three 25-hydroxy vitamin D level subgroups. Three-quarters of the ITP cases had blood vitamin D levels of < 10 ng/mL compared to 64% of the controls. A quarter of the ITP cases had vitamin D levels of (10–20 ng/ml), compared to 36% of the controls. There were no cases or controls with Vitamin D levels of more than 20 ng/ml. The Chi-square test revealed no significant association between vitamin D level and the study group.

Table 2: Frequency and percentage of 25-hydroxy vitamin D based on a normal reference range

Group	25-hydroxy vitamin D level (ng/ml)	
	<10 – No. (%)	10–20 – No. (%)
Patients (n = 63)	47 (75%)	16 (25%)
Controls (n =25)	16 (64%)	9 (36%)

Table 3 shows the mean (± SEM) values of 25 hydroxy vitamin D in the serum according to the phase of disease (newly diagnosed, persistent, or chronic) of cases and controls. The mean values of serum 25-hydroxy vitamin D of the newly diagnosed and chronic phases were not statistically significant, but they were lower than those of the controls. The lowest level of 25- hydroxy vitamin D was found in the chronic phase of the disease. There were also non-significant differences among and between these groups of patients.

Table 3: Mean values (±SEM) of 25-hydroxy vitamin D concentrations of the ITP cases groups and controls

Parameter	ITP Groups			Control (n=25)
	Newly diagnosed (n=21)	Persistent (n=24)	Chronic (n=18)	
25-hydroxy vitamin D (ng/ml)	9.5±1.84	11.4±1.92	8.0±1.13	10.0±1.32

NS: Non- significant (p>0.05)

Table 4 shows the mean (± SEM) values of 25-hydroxy vitamin D concentrations in the serum according to the type of treatment. The mean values of the prednisolone group, prednisolone, and IVIG, prednisolone and mycophenolate group, and romiplostim group were lower than the controls, but they did not reach a significant level. No significant differences were found across the analyzed patient subgroups.

The results also revealed that receiver operating characteristic (ROC) and area under the curve (AUC) for 25-hydroxy vitamin D in differentiation between acute ITP children and controls was (AUC=0.67) at cutoff (5.8 ng/ml) with (sensitivity=52.4, specificity=92.0). Similarly, 25-hydroxy vitamin D has AUC=0.64, at cutoff (6.3 ng/ml) with (sensitivity=55.6, and specificity=80.0) in differentiation between chronic ITP children and controls. Serum 25-hydroxy vitamin D has a significant positive correlation with platelet counts in the Romiplostim group (r=0.51, p=0.032).

Table 4: Mean (±SEM) values of 25-hydroxyvitamin D concentration according to the type of treatment

Parameter	Romiplostim (n=18)	Prednisolone only (n=19)	Prednisolone + IVIG, or prednisolone + mycophenolate (n=26)	Control (n=25)
25-hydroxy vitamin D (ng/ml)	8.2±0.96	8.1±0.96	7.7±0.81	10.0±1.32

Discussion:

The female predominance in the ITP cases of the current study is consistent with that observed by Shaheen *et al.* and Čulić *et al.*, who reported that females were predominant among ITP children in their group (14,17). They found that the mean age of the ITP children was 6.69 years, which agrees with the present study. The blood 25-hydroxy vitamin D level is the most reliable biochemical indicator of vitamin D status, as it reveals the amount of vitamin D that the body makes from the diet, sunlight, and the conversion of vitamin D reserves by the liver (17). 1,25(OH)2D3 exerts its effects by attaching to the vitamin D receptors (VDR). Evidence suggests that VDR is not only found in the colon, bones, and kidneys. also the peripheral blood monocytes and activated lymphocytes. Thus, VDR is recognized to participate in several immunomodulatory functions (18). Matinkia et.al. reported that Vitamin D may be used as a novel immunomodulatory treatment for people with ITP. Thus, there is a supplementary benefit for Vitamin D in individuals with ITP (19). Petrovic et al. reported that most of the ITP children had low levels of vitamin D. which is consistent with the findings of the current study that most of the ITP children had serum 25-hydroxy vitamin D levels below 10 ng/ml (10). Hypovitaminosis D affects the severity of ITP in children at the time of diagnosis, and therapy with vitamin D might be a new possible alternative for ITP treatment (10).

The current study looked at 25-hydroxy vitamin D blood levels in children with ITP at various phases of the illness and found no statistically significant differences. Similarly, Shaheen *et al.* found no statistically significant differences in the mean blood vitamin D concentration between the control group, patients with persistent and chronic ITP, and those newly diagnosed with ITP, even after adjusting for age (17). Lui *et al.* provided an analogy (20) with no significant difference in blood vitamin D levels between healthy controls and ITP patients. In contrast, Čulić *et al.* found that chronic ITP patients had significantly lower blood vitamin D levels compared to acute ITP patients. This was explained by the fact that ITP therapy also reduced their 25(OH)D values (14).

The non-significant differences in 25-hydroxy vitamin D blood levels among types of treatment of ITP in the current study may be due to that all groups began with prednisolone use. No published reports were found about this issue. The osteoblastic and osteoclastic processes that result in the equilibrium of minerals and vitamin D may be impacted by the glucocorticoid treatment of ITP (13). Of note, the levels of platelets reported in the cases were taken at a single point of time during treatment, many of them were normal in terms of response to treatment.

Vitamin D is used as a new medicinal method when high amounts of IFNγ are implicated in the development of illnesses. Individuals just diagnosed with ITP, as well as those who have been living with the ailment for an extended period may find Vitamin D therapy advantageous (21).

Limitation: Inability to include newly diagnosed children with ITP because of limited cases encountered during the time of the study.

Conclusion:

In children who have immunological thrombocytopenia, vitamin D deficiency is prevalent and may be very severe, particularly in the chronic phase.

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Authors' declaration:

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in the place where the research was conducted or samples collected and treated) according to the code number (107) on (18/ 4/ 2024).

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Authors' contributions:

The manuscript should mention the contribution of each author to the research done:

Study conception & design: (Basil O Saleh, Hasanein H Ghali). Literature search: (Huda K Abbas). Data acquisition: (Huda K Abbas). Data analysis & interpretation: (Huda K Abbas & Basil O Saleh). Manuscript preparation: (Basil O Saleh, Hasanein H Ghali & Huda K Abbas). Manuscript editing & review: (Basil O Saleh, Hasanein H Ghali).

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مدى تكرار نقص 25 هيدروكسي فيتامين د لدى الأطفال المصابين بتكسر الصفيحات الدموية المناعي: مرحلة المرض وتاثير نوع العلاج

هدى خضير عباس احمد[1]،باسل عويد محمد صالح [1]، حسنين حبيب غالي[2]
[1]فرع الكيمياء الحياتية السريرية، كلية الطب، جامعة بغداد ، بغداد، العراق.
[2]فرع طب الاطفال ،كلية الطب، جامعة بغداد، بغداد، العراق.

خلفية البحث: يمكن لنقص فيتامين د أن يسبب إضطرابات مناعية في تطور نقص الصفيحات المناعي المزمن.
الأهداف: تحديد معدل تكرار 25 هيدروكسي فيتامين د ذو مستوى منخفض في الأطفال الذين لديهم تكسر الصفيحات الدموية المناعي وأيضا لتقييم تاثير المرض ومرحله ونوع العلاج على مستوى فيتامين د.
الحالات والمنهجية: تم إجراء دراسة الحالات والشواهد هذه في مستشفى حماية الأطفال التعليمي في مدينة الطب، قسم الكيمياء الحياتية السريرية في كلية الطب / جامعة بغداد بين شهري نوفمبر 2023 وأبريل 2024. شملت الدراسة 88 طفلا، 63 منهم تم تشخيص إصابتهم سابقا بنقص الصفيحات المناعي و25 طفلا يتمتعون بصحة جيدة كمجموعة تحكم. تم تقسيم الأطفال الذين يعانون من نقص الصفيحات المناعي إلى مجموعات فرعية، وفقا لمستويات فيتامين د في الدم إلى ثلاث مجموعات أقل من 10 نانوغرام/مل، 10 -20 نانوغرام/مل، 30- 20 نانوغرام/مل. كانت هناك تصنيفات فرعية للأطفال الذين يعانون من نقص الصفيحات المناعي وفقا لمراحل المرض لديهم: حاد، ومستمر، ومزمن. تم قياس مستوى -25هيدروكسي فيتامين د في الدم باستخدام تقنية الاليزا.
النتائج: كشفت النتائج أن غالبية الأطفال ITP (75٪) لديهم مستوى 25 هيدروكسي فيتامين د في الدم أقل من 10 نانوجرام / مل. كانت القيم المتوسطة (± SEM) لمصل 25 هيدروكسي فيتامين د لدى أطفال ITP في المراحل الحادة والمزمنة أقل من تلك الخاصة بالضوابط، لكنها لم تصل إلى مستوى مهم. علاوة على ذلك، كانت قيم 25 هيدروكسي فيتامين د في المصل لدى الأطفال ITP أقل من تلك الخاصة بالضوابط، بغض النظر عن نوع العلاج.
الإستنتاجات: في الأطفال الذين يعانون من نقص الصفيحات المناعي، يكون نقص فيتامين د شائعا وقد يكون شديدا جدا، وخاصة في المرحلة المزمنة. قد يلقي الارتباط الإيجابي الكبير بين 25 هيدروكسي فيتامين د وعدد الصفائح الدموية في مجموعة روميبلوستيم الضوء على نهج العلاج الجديد لهذا المرض.
مفتاح الكلمات: فرفرية نقص الصفيحات الدموية المناعي، 25 هيدروكسي فيتامين د، نقص الصفيحات المناعي، المناعة الذاتية.