

## Study of Vitamin D Receptor Levels in Children with Immune Thrombocytopenic Purpura

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Vitamin D, affecting many tissues and organs of the body. It exerts many of its effects through contact with Vitamin D receptor (VDR) It is important especially in immune system; Immune thrombocytopenia is one of the most common causes of symptomatic thrombocytopenia in children.

**Aim:** The main objective of our study was to study vitamin D receptor level in children with immune thrombocytopenic purpura and effect of vitamin D supplementation upon the response of the thrombocytopenia to conventional therapy of ITP.

**Subjects and Methods:** This is a case control study which included 30 ITP children, who would be attendants to Hematology and Oncology Unit, Pediatric Department, Tanta University Hospital in Egypt. This study would include also 30 apparently healthy children matched in age and sex as a control group. The duration of the study ranges from 6 to 12 months, for all patients and controls the following were done: complete blood count, bone marrow aspiration, serum level of Vitamin D receptors, serum calcium level, serum phosphorus level, serum alkaline phosphatase level and serum parathyroid hormone level.

**Results:** the results revealed that mean  $\pm$  SD of Vitamin D receptors in case group is  $132.43 \pm 14.58$  and there was statistical difference between groups regarding Vitamin D receptor. There was statistical difference between platelets count and Serum total Ca (mg/dl) with negative correlation, while there was no statistical difference between platelets count and Vitamin D receptor. There was statistically significant difference in platelets count in patient with ITP before and after conventional treatment and platelets count in patient with ITP under conventional treatment plus vitamin D supplementation.

**Conclusion:** VD receptors elevation is very common in ITP. Supplementing VD might diminish recurrence. Further research is needed.

*Keywords: Vitamin D receptor; thrombocytopenic; ITP; case control; children.*

## 1. INTRODUCTION

Isolated thrombocytopenia (platelet count 100,000/mms with normal white blood cell count and haemoglobin) characterises immune thrombocytopenia (ITP) in children. In most instances, the aetiology of ITP is unclear, although it may be caused by a viral infection or other immunologic or environmental factors [1].

Autoantibodies against glycoproteins (GP) IIb/IIIa and GPIb/IX present in the platelet membrane cause this immune-mediated illness. ITP is clinically classified into three types, according to recent guidelines from the International Working Group: newly diagnosed (for all cases at diagnosis), persistent (for cases with ITP lasting between 3–12 months from diagnosis, spontaneous remission is not achieved after removal of treatment in between 3–12 months from diagnosis), and chronic (for cases with ITP lasting longer than 12 months from diagnosis, spontaneous remission is not achieved after removal of treatment in between 3–12 months from diagnosis) (lasting for more than 12 mo) [2].

In children, ITP is one of the most frequent causes of symptomatic thrombocytopenia. ITP is predicted to affect between 1 and 6.4 children per 100,000 per year [3]. In an otherwise healthy kid, ITP usually manifests as a petechial rash, bruising, and/or bleeding [3].

Rather than attaining physiological platelet levels, treatment methods focused at restoring platelet counts consistent with acceptable hemostasis. Inhibition of autoantibody synthesis and platelet disintegration are the first-line therapies, whereas immunosuppressive medicines like Rituximab and splenectomy are the second-line treatments. Finally, TPO-receptor agonists are used in third-line therapies to

promote platelet formation by megakaryocytes [4].

Vitamin D is a fat-soluble vitamin that is found in just a few foods naturally. Vitamin D is produced by the skin when exposed to sunlight; however, vitamin D in diet is physiologically inactive and requires two hydroxylations in the body to activate. The first takes place in the liver, when vitamin D [25(OH)D], also known as calcidol, is converted. The second happens mainly in the kidney and produces 1,25 dihydroxy vitamin D [1,25(OH)2D], commonly known as calcitriol, which is physiologically active [5].

The non-calcemic function of vitamin D (VD) in modulating immunological and inflammatory responses has gotten a lot of attention in recent years. Vitamin D nuclear receptor (VDR) is expressed on immune cells, and they can metabolise vitamin D. This hormone is a secosteroid (a steroid with an open B ring) having more functions than only calcium homeostasis. Its activity, like that of other steroid hormones, is mediated by the VDR, a member of the steroid and thyroid hormone receptor superfamily [6].

The calcitriol receptor, also known as VDR or NR111 (nuclear receptor subfamily 1, group 1, member 1), is a transcription factor that belongs to the nuclear receptor family [5]. Calcitriol, the active form of vitamin D, attaches to the VDR, which then joins the retinoid-X receptor to create a heterodimer. This attaches to hormone response sites on DNA, causing particular gene products to be expressed or transrepressed. The VDR is engaged in microRNA-directed post-transcriptional processes as well as transcriptional responses. The VDR gene encodes the vitamin D receptor in humans [7].

## 2. PATIENTS AND METHODS

This study is a case control study which will include 30 children with ITP, who will be attendants to Hematology and Oncology Unit, Pediatric Department, Tanta University Hospital in Egypt from 13 March 2019 to 7 July 2019 This study will include also 30 apparently healthy children matched in age and sex as a control group.

### 2.1 Patients Group Sub-divided into Two Groups

**First:** ITP group under conventional therapy (n= 15).

**Second:** ITP group under conventional therapy with vitamin D (n= 15).

**Inclusion criteria:** Children aged 2 - 18 years with ITP.

**Exclusion criteria:** Children with other causes of thrombocytopenia. Children with ITP on Vitamin D supplementation.

**Methods:** All patients in this study will be subjected to the following:

**A- full history-taking with special emphasis on:** History of upper respiratory tract infections before the beginning of the disease, date of disease onset, duration of disease, history of recurrence, detailed nutritional and developmental history and history of vaccination before the onset of the disease.

**B - Clinical examination with special emphasis on:** General look and appearance of the child, Important signs including Heart rates, breathing rates, pressure of the blood and temp., Anthropometrical measures including body mass, tallness, head perimeter, mid upper arm

circumference, Complete systemic examination including chest, heart, central nervous system and abdomen with especial comment on liver and spleen state, Skin, soft tissues, LNs for Purpura, Ecchymosis and Lymphadenopathy, Clinical examination for different presentation of immune thrombopenia: Purpura, Ecchymosis and Mucous membrane bleeding and Clinical examination for different clinical manifestations of vitamin D deficiency and rickets Broad epiphysis, Ricketic rosaries, Box shaped head, Bow legs, Marfan sign, Genue varum deformity, Pigeon breast, Frontal bossing and Delayed teething.

**C- Laboratory investigations including:** Complete blood count including platelet, Bone marrow aspiration, Serum level of 25 hydroxyvitamin D receptors, calcium, phosphorus, Alkaline phosphatase and Parathyroid hormone.

**Administrative and Ethical Design:** A written clear agreement was got from the all involved case's parents. The current work had been permitted by the ethics committee on research concerning human subjects of Medicine Faculty, Tanta University.

**Data management and Statistical Analysis:** Collected data were recorded then presented and analyzed statistically by computer using SPSS-22 (SPSS Inc. Chicago, IL, U.S.A) as follow: Editing and coding, Data entry in computer. Data were summarized and presented in tables and graphs and summarized as median and mean  $\pm$  standard deviation (SD) for quantitative variables and as numbers and percentages for qualitative variable.

## 3. RESULTS

This table shows significantly high serum level of vitamin D receptors in studied patients compare with controls.

**Table 1. Comparison between the two studied groups according to specific data**

Specific data	Patients (n=30)	Controls (n=30)	t	P
Vitamin D receptor				
Min. – Max.	97.0 – 159.0	93.0 – 252.0	2.909*	0.007*
Mean $\pm$ SD.	132.43 $\pm$ 14.58	513.30 $\pm$ 716.95		
Median (IQR)	131.0(122.0 – 145.0)	122.0(117.0 – 597.0)		

t: Student t-test

p: p value for comparing between the studied groups

\*: Statistically significant at  $p \leq 0.05$

**Table 2. Correlation between platelet count and specific data in patient group**

Specific data	Platelet count	
	R	P
Vitamin D receptor	-0.098	0.607
Serum total ca (mg/dl)	-0.371	0.044*
Serum Mg (mg/dl)	0.179	0.345

*r*: Pearson coefficient

\*: Statistically significant at  $p \leq 0.05$

**Table 3. Comparison between the two studied groups according to platelet count before and after treatment**

Platelet count ( $\times 10^3$ )	Before treatment	After treatment	t	P
ITP group under conventional therapy (n= 15)				
Min. – Max.	6.0 – 78.0	45.0 – 111.0	6.526*	<0.001*
Mean $\pm$ SD.	44.93 $\pm$ 23.74	79.60 $\pm$ 20.27		
Median (IQR)	45.0	77.0		
ITP group under conventional therapy with vitamin D (n= 15)				
Min. – Max.	14.0 – 70.0	70.0 – 156.0	9.907*	<0.001*
Mean $\pm$ SD.	49.93 $\pm$ 17.29	107.87 $\pm$ 25.30		
Median (IQR)	60.0	103.0		
$p_1$	0.515	0.002*		

*t*: Paired t-test

*p*: *p* value for comparing between before and after

$p_1$ : *p* value for Student t-test for comparing between the studied groups

\*: Statistically significant at  $p \leq 0.05$  vitamin D is given at dose of 3000 IU per day for one month then platelet count was measured after one week from last dose

This table show that insignificant negative correlation between platelets count and vitamin D receptor, while there was significant negative correlation between platelets count and Serum total Ca (mg/dl).

This table demonstrates that platelet counts in patients with ITP before and after conventional therapy, as well as platelets counts in patients with ITP who received conventional treatment with vitamin D supplementation, differed statistically significantly.

#### 4. DISCUSSION

The mean SD of Vitamin D receptors in the case group was 132.43 14.58 in the present research, and there was a significant difference between groups when it came to Vitamin D receptors. In agreement of our result Fattizzoet al. [8] found that in autoimmune cytopenias and hematological disorders, specifically ITP, Evan's VDR expression levels were higher in the disease state compared to normal donors. Overall, the majority of cells implicated in

haematological disorders strongly express VDR. This indicates that these cells may respond to vitamin D therapy, which may result in anti-tumor actions. The capacity to restore VDR levels in illnesses with low VDR levels should be studied in order to improve the sensitivity of these cells to vitamin D therapy [9].

Baeke et al. [10]. Described that activated T-cells have high levels of VDR while naïve T-cells have very low or undetectable levels of VDR. T-cell activation raises VDR and CYP27B1 levels, which peak 48 hours after activation, in contrast to CYP24A1, which is not detectable during isolation or after activation. Platelets count and serum total calcium (mg/dl) had a significant difference with a negative association, while platelets count and Vitamin D receptor had no statistical difference. Ahmed et al., [11] Found that the mean platelet count at time of diagnosis was calculated in 12 studies. Patients with chronic ITP had a substantially higher platelet count upon diagnosis, with a mean difference of 5.27. (95 percent CI 2.69-7.86).

Yildirmak et al. [12] Found a significantly higher mean MPV of 9.2 fL in patients developing chronic ITP compared with a mean of 8.1 fL in patients with recovered ITP. In agreement with our results, Park et al. [13] investigated whether vitamin D deficiency is associated with increased platelet count (PC) and mean platelet volume (MPV) and they found that platelet count was inversely related to vitamin D levels. In individuals with vitamin D insufficiency, no prior research has convincingly shown an increase in platelet count. The tight connection between oxidative stress and platelet count may explain these findings.

Our results are in agreement with study of [14-15] as they found that there was a highly statistically significant difference between the ITP group and the control group regarding platelet count.

Our study showed that there was statistically significant difference in platelets count in patient with ITP before and after conventional treatment and platelets count in patient with ITP under conventional treatment plus vitamin D supplementation. Antico et al. [16] speculated that VD at high doses can affect the development or even the symptoms of the autoimmune diseases. As a result, several of the writers advocated adopting VD as a new therapeutic option for ITP. However, the majority of the writers believe that additional research, particularly randomized clinical trials, is required to better understand the impact of VD on autoimmune disorders.

## 5. CONCLUSION

Vitamin D Receptors (VDR) increase is extremely frequent in children with Immune thrombocytopenic purpura, according to the findings of this research (ITP). Furthermore, the impact of vitamin D intake on the response of thrombocytopenia to standard ITP treatment may help to prevent recurrence. However, more study is needed.

## 6. RECOMMENDATIONS

- Further studies on large geographical scale and on larger sample size to emphasize our conclusion.
- Further studies to investigate the connection between Vitamin D receptor

level, the incidence, severity and chronicity of ITP.

- A prospective randomized placebo-controlled trials needs to be performed to investigate the role of VD as an immune-modulating drug for patients with ITP.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT AND ETHICAL APPROVAL

The present study was conducted on 50 children after the approval of the ethical committee, and consent from all studied groups.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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