TaqI Polymorphism, Vitamin D Receptor Gene mRNA Expression and Vitamin D Levels in Autoimmune Thrombocytopenia

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Received: 13 July 2025

Accepted: 23 August 2026

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DOI 10.5001/omj.2026.80

Abstract

Objectives: Secondary thrombocytopenia is believed to be associated with polymorphism in the Vitamin D receptor (VDR) gene, which is caused by the complicated and diverse underlying pathomechanisms of Idiopathic thrombocytopenic purpura (ITP). This study aims to examine the Taql polymorphism, VDR gene mRNA expression, and vitamin D levels in individuals with primary and secondary autoimmune thrombocytopenia.

Methods: By using the enzyme-linked fluorescence assay (ELFA), rt-PCR, and restriction fragment length polymorphisms (RFLP) PCR, all eligible subjects were evaluated for their vitamin D levels, VDR gene mRNA expression, and Taql polymorphism, respectively. The study included 40 samples of both primary and secondary autoimmune thrombocytopenia (1:1 ratio).

Results: Primary autoimmune thrombocytopenia had substantially lower vitamin D levels than secondary autoimmune thrombocytopenia (19.26 ng/mL vs. 25.14 ng/mL, $p<0.05$). Primary autoimmune thrombocytopenia had considerably lower VDR gene mRNA expression than secondary autoimmune thrombocytopenia (9.53 vs. 12.09, <p0.05). TaqI polymorphisms weren't identified in either of the two groups, albeit.

Conclusions: primary autoimmune thrombocytopenia is closely related to low VDR gene mRNA expression but has not yet been elucidated its association with VDR gene TaqI polymorphisms.

Keywords: Primary autoimmune thrombocytopenia, secondary autoimmune thrombocytopenia, Vitamin D levels, VDR mRNA expression, VDR gene polymorphisms.

Introduction

Autoimmune thrombocytopenia is a bleeding disorder caused by an autoimmune response, which results in a low platelet count of less than 100,000 cells/uL. The condition can occur due to various reasons, such as inadequate production of platelets, increased destruction of platelets, sequestration in the spleen, or abnormal distribution. Primary autoimmune thrombocytopenia and secondary autoimmune thrombocytopenia are the two categories into which autoimmune thrombocytopenia can be subdivided. Autoimmune thrombocytopenia is caused by the glycoproteins that are found on the surface of platelets. Malignancy, the use of certain medications (chemotherapy, radiation, aspirin, and ibuprofen), exposure to specific substances (arsenic, benzene, and pesticides), or the secondary immune effects of chronic illnesses like autoimmune diseases and infections like hepatitis, HIV, and Rubella can all contribute to secondary autoimmune thrombocytopenia.[1,2]

Antiplatelet autoantibodies against glycoproteins (GP), particularly GPIIb/IIIa or GPIb, that result in increased platelet lysis are associated with an increased risk of autoimmune thrombocytopenia. Auto-reactive B lymphocytes producing antiplatelet antibodies are the primary aetiology of ITP. Autoantibodies were not found in 30-40% of ITP patients. The antiplatelet autoantibodies are under the control of T lymphocytes. Increased CD11a expression in CD3+T lymphocytes of ITP patients may contribute to resistance to immunosuppressive therapy. [3,4]

Vitamin D is an important immune regulator that prevents the immunosuppressive effect of PD-L1,[5] as the latter is responsible for T cell activation, proliferation, and cytotoxicity.[6] Toll-like receptors 2 and 4 (TLR2 and TLR4) play a considerable role in the host defence against microorganisms. Vitamin D's immunomodulatory effects downregulate them.[7,8] A significant relationship between TGF-β, a key regulator of immune homeostasis,[9] and vitamin D deficiency is established.[10] Also, interleukin-10 (IL-10) is a key regulator of immune homeostasis and can predict the clinical course of ITP, as it is significantly higher at the onset of disease.[11–13]

Various VDR genetic variants have been reported in different diseases and populations. TaqI is a genetic polymorphism that has been linked to the development of multiple medical conditions, including SLE, multiple sclerosis, type II diabetes mellitus (DM), and tuberculosis, among others. Its identification in these diseases highlights the potential for genetic testing to aid in the diagnosis and treatment of complex medical conditions.[14] Several studies indicated that the TaqI polymorphism increased the Th1 and Th2 lymphocyte cell ratio in tuberculosis infection.[15] TaqI and ApaI polymorphisms have been found to play a role in several autoimmune diseases and correlate with vitamin D levels. In 2018, Bae reported that TaqI and ApaI polymorphisms were associated with the severity and clinical symptoms of SLE patients.[16]

The TaqI polymorphism is the most common in Asia, accounting for 77% of studies in Japan and India, and it correlates with bone density. TaqI polymorphism is also strongly associated with vitamin D3 levels. This is because the TaqI polymorphism affects calcium metabolism, a key component of the feedback mechanism that regulates vitamin D levels.[17,18] However, to date, no research on VDR polymorphisms in Indonesia has been published.

Research on autoimmune diseases with VDR gene polymorphisms and Vitamin D deficiency is still being debated. Investigations are still ongoing to determine whether the VDR gene polymorphism affects Vitamin D deficiency and makes it easier for autoimmune diseases to develop, or vice versa. Of particular interest is how Vitamin D affects VDR signaling and the expression of VDR gene mRNA, which causes autoimmune thrombocytopenia, including in Indonesia. Through a pathway involving vitamin D, this study attempts to differentiate between the pathomechanisms of primary autoimmune thrombocytopenia and secondary autoimmune thrombocytopenia.

Methods

This study's design was observational analytical, and it conducted from August 2019 to July 2020. Adult patients with autoimmune thrombocytopenia with a platelet count under 100,000 cells/L made up the study sample. The specimens were analyzed in the Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, and the Clinical Pathology Laboratory Installation at dr. Wahidin Sudirohusodo Hospital Makassar, where the research sample was collected.

Examination of vitamin D levels using the ELFA method, Examination of mRNA expression of the VDR gene using the rt-PCR method, and expressed in units of Fold Change. Examination of TaqI Polymorphism in Exon9 (352T/C) using the Restriction Fragment Length Polymorphisms (RFLP) PCR method, TaqI enzyme cleavage at 252bp and 748bp. On 26 July 2019, this research met all necessary

requirements and was granted ethical approval by the Health Research Ethics Commission of the Medical Faculty at Hasanuddin University, as well as the State University Hospital of Hasanuddin University and the Dr. Wahidin Sudirohusodo Hospital in Makassar. The approval number for this research was 557/UN4.6.4.5.31/PP36/2019.

This process was performed on isolated DNA samples. First, a PCR mix was prepared with 22.5 µl of specific primers for the VDR gene to be amplified: 2.5 µl of 10X PCR buffer, 0.5 µl of Taq polymerase, 2 µl of MgCl2, 2 µl of dNTPs, and 13.5 µl of distilled water. Then, 1 µl of each primer (F primer: **ctggggagcggggagtatgaagga**) and 1 µl of primer (R primer: **gggtggcggcagcggatgta**) are added. Next, 2.5 µl of DNA extract was added to 22.5 µl of PCR primer (F/R) mixture, and amplification was carried out with a first step at 94°C for 2 minutes, followed by 40 cycles of 60 seconds at 94°C, 45 seconds at 60°C, 1 minute at 60°C, and 60 seconds at 72°C. This process was continued at 72°C for 2 minutes using a PCR machine (Applied Biosystem 2720 Thermal cycler). After that, 2.5 µL of the amplified product was added to a loading buffer mixture for electrophoresis.

PCR product analysis and interpretation by gel electrophoresis. Each 10 µl of amplified product was mixed with 5 µl of loading solution. After thorough mixing, each well was loaded into a 2% agarose gel well, submerged in a tank containing TBE buffer. Electrophoresis was then run for 1 hour at a constant voltage of 75 volts. After 1 hour, electrophoresis was stopped, and the gel was removed for UV examination. Interpretation of the results, including positive for the presence of DNA bands and negative for the absence of DNA bands.

For the restriction fragment length polymorphisms (RFLP) PCR analysis, the amplicon of the VDR gene locus was cut with a specific cutting enzyme, TaqI (Fermentas, USA), according to the Fermentas manufacturer's protocol. If polymorphism was present, the PCR products after cutting with the TaqI enzyme were 252 bp and 748 bp, respectively. If polymorphism was absent, the PCR product was 1100 bp, as determined by electrophoresis and a 2.5% agarose gel stained with ethidium bromide.

Normalization and fold-change calculation method for VDR mRNA expression. Specific primer oligonucleotides were used for the VDR and GAPDH genes as housekeeping genes (internal controls). Detecting mRNA VDR genes using the following primers: (1) VDR for CTC ATC TGT CAG AAT GAA CTC CTT CA; (2) VDR Rev for TCA CCA AGG ACA ACC GAC G; (3) GAPDH for GAA GGT GAA GGT CGG AGT CA; (4) GAPDH Rev for CTT TAG GGT AGT GGT AGA AG. Calculation methods used an expression formula (Slope*Log template + Starting Quantity = Fold Change).

Results

There were 40 samples in this study which were divided into two groups, namely primary autoimmune thrombocytopenia (20 samples) and secondary autoimmune thrombocytopenia (20 samples). Table 1 shows the variations in characteristics between the two groups. The distribution of the gender and age groups did not differ significantly. Platelet count was found to be significantly lower ($p<0.05$) in primary autoimmune thrombocytopenia ($38.85x10^3$ cells/ul) than in secondary autoimmune thrombocytopenia ($66.30\ x10^3$ cells/ul. Vitamin D levels in primary autoimmune thrombocytopenia were substantially lower (19.26 ng/ml) than in secondary autoimmune thrombocytopenia (25.14 ng/mL). VDR gene mRNA expression was significantly lower in primary autoimmune thrombocytopenia (9.53) than in secondary autoimmune thrombocytopenia (12.09).

Table 1: Differences in characteristics and biomarkers between primary and secondary autoimmune thrombocytopenia.

Variable	Primary autoimmune thrombocyte-penia (n=20)	Secondary autoimmune thrombocyte-penia (n=20)	p-value
Gender			
Male	5 (12.5%)	4 (10%)	1.000[a]
Female	15 (37.5%)	16 (40%)	

Age (years), Min-Max	19-69	22-68	
Mean (SD)	37.8 (164)	41.9 (15.4)	0.420[b]
<60	17 (42.5%)	16 (40%)	
≥60	3 (7.5%)	4 (10%)	
Platelet count (10^3sel/uL), Min-Max	2-96	19-99	
Mean (SD)	38.85 (29.73)	66.30 (27.50)	0.004[b,*]
Vitamin D levels, Mean (SD) (ng/ml)	19.26 (8.54)	25.14 (8.85)	0.039[b,*]
Vit D mRNA expression, Mean (SD) (fold change)	9.53 (0.57)	12.09 (0.65)	<0.001[b,*]

[a]Chi-square test, [b]Independent t test, *significant at p<0.05.

The analysis results in Table 2 show that Vitamin D levels correlate significantly (p<0.05) with platelet count, indicating that higher serum Vitamin D levels are associated with higher platelet counts, with a moderate correlation strength (r=0.407) in secondary autoimmune thrombocytopenia. In the primary autoimmune thrombocytopenia group, no significant relationship was found between Vitamin D levels or VDR mRNA expression with platelet count (p>0.05). As depicted in Figure 1, the correlation was weak and not significant in both variables. VDR mRNA expression did not show a significant correlation in this group.

Table 2: Correlation between Platelet Count, Serum Vitamin D Levels, and the Expression of the VDR Gene in Primary and Secondary Autoimmune Thrombocytopenia.

Correlated Variable	Platelet Count			
	Primary autoimmune thrombocytopenia (n=20)		Secondary autoimmune thrombocytopenia (n=20)	
	Bivariate	Partial	Bivariate	Partial
Vitamin D levels[a]	r= - 0.096 p=0.344	r= - 0.031 P=0.453	r= 0.451 p=0.023	r= 0.407 p=0.047
Vit D mRNA expression[b]	r=0.014 p=0.477	r= - 0.020 p=0.470	r= - 0.335 p=0.073	r= - 0.270 p=0.147

[a]Pearson Correlation, [b]Spearman-Rank Correlation.

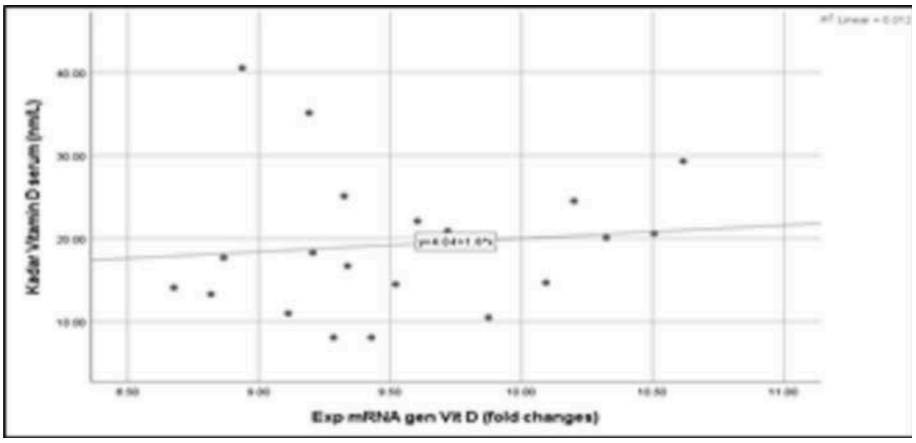

Figure 1: Scatter plot showing correlation between platelet count in thrombocytopenia and Vitamin D Receptor Gene mRNA expression.

Figure 2 shows that of the 40 samples, no bands formed at 252bp and 748bp, only formed at 1100bp. This study found no TaqI polymorphism in primary or secondary autoimmune thrombocytopenia.

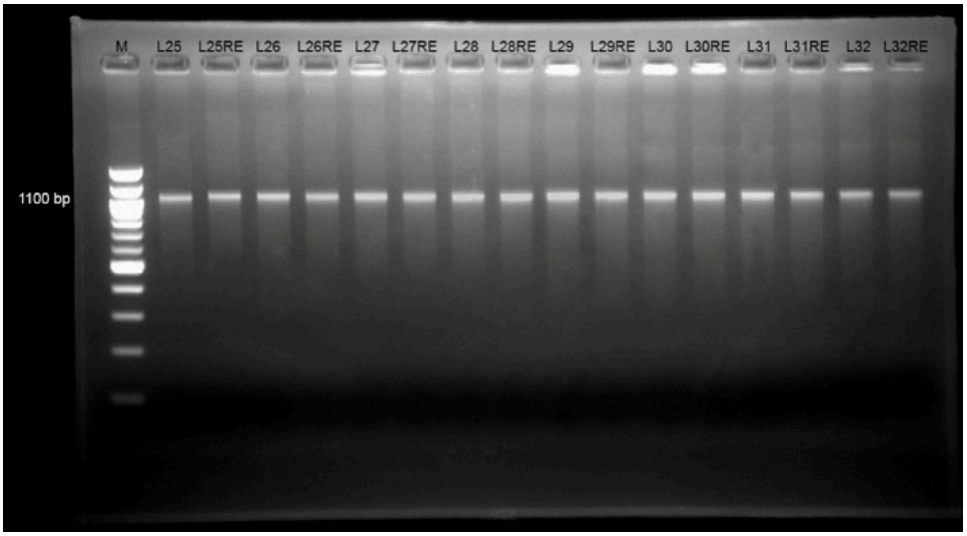

Figure 2: TaqI electrophoretic band description with the RFLP method.

Discussion

The findings of a recent study suggest that patients with secondary autoimmune thrombocytopenia have higher levels of vitamin D compared to those with primary autoimmune thrombocytopenia. The study also indicates a strong correlation between vitamin D levels and platelet counts in patients with secondary ITP. Interestingly, the study observed a positive linear correlation between vitamin D levels and platelet count in secondary autoimmune thrombocytopenia but not in primary autoimmune thrombocytopenia. Furthermore, the study found that increased serum vitamin D levels were negatively correlated with vitamin D receptor gene mRNA expression, which could explain the inverse correlation between serum vitamin D levels and platelet count in secondary autoimmune thrombocytopenia. Stated differently, individuals with secondary autoimmune thrombocytopenia exhibit a negative correlation between the expression of the Vitamin D receptor gene mRNA and platelet count. These results clarify the intricate connection between autoimmune thrombocytopenia and vitamin D.

Individual variations in vitamin D levels are possible due to the body's spontaneous synthesis of the vitamin. Vitamin D levels were observed to be greater in patients with secondary ITP than in patients with primary ITP. Interestingly, in patients receiving immunomodulatory drugs that acted on monocytes, macrophages, dendritic cells (DCs), T lymphocytes, and B lymphocytes to elicit an effect on both innate and adaptive immune responses, these levels did not correlate with vitamin D levels. Moreover, autoimmune illnesses are at a lower risk in people who use vitamin D. According to several studies, autoimmune disease patients' serum VD levels are much lower than those of healthy people. [19]

Vitamin D deficiency and ITP were associated. Prednisone had no effect on the platelet count, but Vitamin D and hydroxychloroquine did. Yet, it is still certain that the mechanism needs to be found.[20] A study by Culic revealed a significant association between Vitamin D levels and both acute and chronic ITP, as opposed to platelet counts.[21] Compared to healthy individuals, both ITP patients and non-ITP patients with thrombocytopenia had lower levels of Vitamin D. This suggests that Vitamin D may play a role in the development of ITP, as evidenced by the lower levels of the vitamin in ITP patients compared to healthy individuals.[22]

Serum levels of vitamin D, vitamin D receptors (VDR), and T helper cells (TH1, TH2, and TH17) in individuals with immunological thrombocytopenia (ITP) have been examined in a number of investigations. Because anti-erythrocyte autoantibodies have developed, vitamin D levels in ITP patients may be much lower than in controls. In addition, it was discovered that ITP patients had reduced VDR levels. Low platelet counts in ITP patients were associated with decreased levels of vitamin D. It is interesting to note that in ITP patients, the generation of anti-erythrocyte autoantibodies was decreased at 10, 20, and 40 ng/mL of vitamin D. These results imply that vitamin D may have an immunomodulatory

function in people with immune-related disorders and also in those with other autoimmune diseases that cause a weakened immune response. Moreover, there is a direct link between the incidence and clinical manifestation of autoimmune and hematological disorders and vitamin D deficiency. Therefore, by treating the immunological abnormalities that contribute to the formation of chronic ITP, vitamin D treatment may help modify the immune system and lower the risk of developing it.[21]

The relationship between oxidative stress and platelet count may explain the unfavorable link observed between vitamin D levels and platelet count. The cytokines IL-6 and TNF, which regulate thrombopoiesis and inflammation, are released in excess when there is a vitamin D shortage, which raises mean platelet volume (MPV). Vitamin D is an antioxidant that helps inhibit the release of vascular endothelial growth factors (VEGF) and reduce inflammation, lowering platelet count. Oxidative stress can cause thrombocytosis. Since MPV is thought to be negatively correlated with blood vitamin D levels, maintaining appropriate levels is essential for lowering the risk of illness.[23]

In the current study, primary autoimmune thrombocytopenia had considerably lower VDR gene mRNA expression than secondary autoimmune thrombocytopenia. In contrast, neither primary nor secondary ITP patients show a correlation between the VDR gene mRNA expression and vitamin D levels or platelet counts. In 2014, Handono discovered that SLE patients with low amounts of vitamin D also had low levels of VDR gene expression, albeit this did not associate with the severity of the condition.[24] Agrawal *et al.* discovered various facts, including that the lower the amounts of vitamin D, the less the VDR gene is expressed. Proinflammatory cytokines like TNF- are increased by vitamin D deficiency, which increases inflammation and suppresses the production of the VDR gene. TNF-α can be reduced by vitamin D supplementation, which will lead to increased expression of the VDR gene.[25] Taking 2000 IU of vitamin D for two months raised serum levels by 65% and vitamin D expression by 60 times.[26]

Levels of vitamin D and VDR affect anti-thrombogenicity. Antithrombotic factor gene expression is upregulated by activated vitamin D, and thrombogenic factor gene expression is downregulated by thrombomodulin in monocytes. Numerous health benefits of vitamin D have been discovered for the human body. For example, it may inhibit the circulation's release of inflammatory mediators such as TNF-alpha, ICAM-1, and VCAM-1, which may aid in preventing the activation of TACE in renal cells. Furthermore, vitamin D supplementation has been demonstrated to enhance megakaryopoiesis and decrease TNF-α and IL-6 levels, which are connected to oxidative stress. Moreover, individuals with monocytic leukemia have also been reported to benefit from vitamin D's anticoagulant properties. In general, it is impossible to overestimate the possible health advantages of vitamin D.[27]

Vitamin D interacts with the Vitamin D receptor (VDR), which is found in many tissues, to influence the immune system. As a steroid, vitamin D regulates calcium and bone metabolism by acting as an intracellular receptor for 1,25(OH)2 D. Additionally, 1,25-dihydroxyvitamin D3 [1,25(OH) 2D3], the active form of vitamin D, promotes immunomodulation via VDR on almost all immune cell types, including CD4+ and CD8+ expressed on antigens. Dendritic cells, neutrophils, macrophages, B and T lymphocytes, and macrophages are all activated by the antigen-presenting cell (APC). Because it alters adaptive immune responses and activates innate immunity, vitamin D is essential to this process. The relationship is made either by directly interacting with portions of the promoter regions of cytokine genes susceptible to vitamin D or by interfering with nuclear transcription factors such as NF-AT and NF-KB. The impact of vitamin D on the immune system is substantial and varied overall.[28,29]

The VDR gene is responsible for producing a specific protein that enables the body to respond to vitamin D. Recent research suggests that VDR expression in immune cells might have an impact on the functioning of the immune system. The VDR complex, consisting of 1,25(OH)2D3, VDR, RXR, transcription activation factor (TAF), histone acetylase transferase (HAT), and basal transcription components, can activate or suppress miRNAs, thereby regulating gene expression. This approach uses miRNA, host gene promoter sequence-based direct transcriptional regulation, or other transcription factors-based indirect transcriptional regulation. In contrast, miRNAs may control the action, production, and metabolism of vitamin D or be impacted by signals from the vitamin D hormone receptor in a dynamic feedback system. Synthetic VDR agonists have anti-proliferative, immunomodulatory, differentiation, and anti-inflammatory effects that can be used to treat autoimmune thrombocytopenia and other autoimmune illnesses. Insufficient vitamin D production or intake can reverse the negative effects of reduced VDR function and vice versa.[30]

Cancer and autoimmune illnesses exhibit higher levels of VDR mRNA expression than healthy individuals. Target cell-specific interactions of 1,25(OH)2D3 with particular nuclear receptors (VDR) are associated with primary and secondary autoimmune thrombocytopenia prognostic variables. The complex

formed between 1,25(OH)2D3 and VDR has the ability to influence the effects of vitamin D through transcriptional patterns or posttranscriptional activities. VDR is present in almost all organs, including hematopoietic cells, where it plays a crucial role in suppressing the immunological activity of T and B lymphocytes while stimulating the activation of monocytes and macrophages by 1,25(OH)2D3. It is noteworthy that VDR belongs to the same steroid receptor superfamily as the receptors for thyroid hormone and vitamin A.[31]

Vitamin D has been found to activate the VDR gene during the process of thrombopoiesis, which is the production of platelets. The megakaryoblastic cells have been observed to produce VDR mRNA, and forskolin, a compound that activates the enzyme that differentiates adenylate cyclase, has been found to increase the levels of VDR mRNA. The megakaryocytic maturation process, which is characterized by cell size, nuclear multiplication, increased granularity, and the expression of specific proteins that form platelets, is initiated by the protein kinase C stimulator, phorbol ester 12-O-tetradecanoylphor-bol-13-acetate (TPA), during in vitro megakaryocyte differentiation.[32]

VDR polymorphism is an additional indicator of vitamin D's role in autoimmune disorders. ApaI, BsmI, TaqI, and FokI are the four forms of VDR polymorphisms that have been found and are being researched for their potential involvement in autoimmune illnesses. Although the distinctions in polymorphisms between diseases and their protective effects are unknown, these four polymorphisms have been linked to ethnicity and the onset of autoimmune disorders.[21]

In either primary or secondary autoimmune thrombocytopenia patients, the current investigation identified no Taql polymorphism of the VDR gene. Autoimmune illnesses are more common in people with VDR polymorphisms, particularly BsmI, ApaI, TaqI, and FokI. Low vitamin D has been related to several autoimmune illnesses, including thyroiditis, autoimmune gastritis, rheumatoid arthritis, MS, type 1 diabetes, and SLE. Vitamin 25 (OH) D status and the development of autoimmune disorders were connected with a relative risk of 0.94 (95% CI 0.90-0.98) for autoimmune disease, according to a study done on patients with vitamin D deficiency, whose vitamin D levels were less than ten nmol/L.[19]

Complex interactions exist between SLE risk genes and polymorphisms in the VDR gene. The relationship between VDR BsmI (rs1544410), FokI (rs2228570), ApaI (rs7975232), and TaqI (rs731236) gene polymorphisms and SLE risk was examined in a meta-analysis study that comprised 13 research articles. Overall, SLE risk was associated with the BsmI B allele and bb genotype, the FokI f allele and ff genotype, and the ApaI aa genotype. The FokI f allele, BB genotype, bb genotype, ff genotype, and BsmI B allele are all linked to an elevated risk of SLE in Asia. Similarly, an elevated incidence of SLE has been associated in Africa with the BsmI B allele, BB genotype, bb genotype, FokI f allele, ff genotype, ApaI A allele, AA genotype, and aa genotype. However, the TaqI, BsmI, FokI, and ApaI VDR gene polymorphisms do not raise the risk of SLE in Caucasians. A different study looked into the involvement of group-specific component (GC) genes (rs7041 and rs4588), CYP2R1 (rs10741657), functional VDR polymorphisms (TaqI rs731236, ApaI rs7975232, FokI rs2228570, and BsmI rs1544410), and GC genes in the development of autoimmune thrombocytopenia. The research discovered that the BsmI or TaqI polymorphisms are substantially associated with the risk of autoimmune thrombocytopenia, in contrast to the ApaI or FokI polymorphisms.[19]

This study examined the presence of a particular single nucleotide polymorphism (SNP), known as the TaqI polymorphism (rs731236), in codon 352 (isoleucine) of exon 9, located near the 3' untranslated region of the VDR gene on chromosome 12, in relation to primary and secondary autoimmune thrombocytopenia. However, no TaqI polymorphism was found to be associated with either condition. Although the TaqI polymorphism is a potential candidate, it does not alter the structure of the VDR protein, and therefore, it is unlikely to be a genetic cause of autoimmune thrombocytopenia. Additionally, this study found that vitamin D levels have no influence on the condition.[33]

The TaqI genotype plays a significant role in driving the production of VDR mRNA, whereas ApaI does not have such an impact. Thus, the relationship between ApaI, BsmI, and TaqI polymorphisms is not uniform. These four polymorphisms (FokI, BsmI, ApaI, and TaqI) are strongly linked to the risk of disease progression, which contradicts their relevance to disease. Mononucleotide repeats in the third untranslated region (3'UTR) are also associated with this polymorphism. Although the BsmI and ApaI polymorphisms are located in exon 9, they are intronic (between exons 8 and 9), and none of these three modifications lead to an amino acid substitution. The TaqI polymorphism is also located in exon 9 and does not result in an amino acid substitution. While it has been suggested that these four polymorphisms affect the stability of VDR mRNA, no studies have examined how they affect ligand binding. Researchers have concluded that there is no correlation between VDR mRNA expression and VDR polymorphism, as the two variables have inconsistent relationships.[34]

Our study has several limitations, including not assessing factors affecting VDR gene mRNA expressions and vitamin D levels, such as GPIIb/IIIa Autoantibodies, Thrombopetin, or cytokines (IFN and interleukins). This research also has sample limitations, particularly in examining the TaqI polymorphism. This may be related to the complete absence of TaqI polymorphism in all 40 subjects. The VDR gene polymorphism is limited to the TaqI genotype, which has not seen other polymorphisms.

Conclusion

The study found that primary autoimmune thrombocytopenia was linked to significantly lower levels of vitamin D and VDR gene mRNA expression compared to secondary autoimmune thrombocytopenia. However, there was no significant association found between VDR gene expression and vitamin D levels in individuals with either primary or secondary autoimmune thrombocytopenia. Patients with secondary autoimmune thrombocytopenia are advised to consider vitamin D supplementation, as serum vitamin D levels are significantly associated with platelet count in this condition. The absence of TaqI polymorphisms in primary and secondary autoimmune thrombocytopenia in this study must be confirmed in subsequent studies. This suggestion may be made by assessing factors affecting VDR gene mRNA expressions and vitamin D levels, such as GPIIb/IIIa autoantibodies, Thrombopetin, or cytokines (IFN and interleukins) in patients with autoimmune thrombocytopenia.

Acknowledgments

The authors acknowledge the Farhamna Academic for assisting in the preparation of this manuscript. The authors also acknowledge to the staff of the clinical pathology department, Faculty of Medicine, Hasanuddin University, and Clinical Laboratory of Wahidin Sudirohusodo Hospital Makassar, who have assisted in data collection and acquisition. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Authors' Contributions

AN and MA conceived and designed the analysis, collected the data, contributed data or analysis tools, performed the analysis, and wrote the paper. MH, TH, RN, MNM, TT, UB, RM, IP, AD, and RM conceived and designed the analysis and wrote the paper.

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