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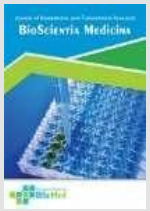

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The Shadow of Deficiency: Vitamin D Status as a Critical Determinant of Antithyroid Drug Efficacy in Graves' Disease – Insights from an Indonesian Cohort

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A B S T R A C T

Background: Graves' disease (GD), an autoimmune hyperthyroid condition, presents significant management challenges, particularly concerning variable remission rates with antithyroid drugs (ATDs). Vitamin D, with its established immunomodulatory properties, is hypothesized to influence autoimmune processes, including those in GD. However, its precise impact on ATD treatment outcomes in diverse populations, especially in regions like Indonesia with high vitamin D deficiency prevalence, remains insufficiently elucidated. **Methods:** This retrospective cohort study was conducted at Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia, analyzing data from 73 newly diagnosed adult GD patients (diagnosed January 2022 - December 2023). Patients had confirmed GD based on hyperthyroidism with orbitopathy or positive TRAb and were followed until May 2025. Baseline serum 25-hydroxyvitamin D [25(OH)D], free T4 (fT4), and TSH levels were recorded. Vitamin D deficiency (VDD) was defined as <20 ng/mL. The primary outcome was non-remission after ATD therapy, defined as failure to achieve stable euthyroidism for ≥6 months on minimal ATD doses. Multivariate Poisson regression was used to assess predictors of non-remission. **Results:** Of 73 patients (mean age 36.2 years; 62% female), 55 (75.3%) exhibited VDD. Multivariate analysis identified VDD as a significant independent predictor of non-remission (adjusted Relative Risk [aRR] 11.81; 95% CI 1.88–74.20; p=0.008). Elevated baseline fT4 levels (≥5 ng/dL; aRR 4.61; 95% CI 1.13–18.70; p=0.032) and older age (>48 years; aRR 0.078; 95% CI 0.06–0.95, indicating a protective effect of older age against non-remission) were also significant predictors. **Conclusion:** Baseline vitamin D deficiency is a potent independent risk factor for non-remission in Indonesian Graves' disease patients receiving antithyroid drug therapy. These findings underscore the potential importance of assessing and addressing vitamin D status in the management of Graves' disease to optimize therapeutic outcomes.

1. Introduction

Graves' disease (GD) stands as the predominant cause of hyperthyroidism globally, an organ-specific autoimmune disorder triggered by the generation of autoantibodies against the thyroid-stimulating hormone receptor (TSHR-Abs or TRAb). These antibodies mimic the action of TSH, leading to uncontrolled thyroid hormone synthesis and secretion, culminating in a state of thyrotoxicosis. The clinical spectrum of GD is diverse, ranging from mild symptoms to severe manifestations such as thyroid storm, ophthalmopathy, and dermopathy,

significantly impacting patients' quality of life. Epidemiologically, GD affects individuals across various age groups but shows a predilection for those aged between 20 and 49 years, with a marked female predominance. The incidence is estimated at 20–50 cases per 100,000 individuals annually, making it the second most common endocrine disorder after diabetes mellitus. Untreated or inadequately managed GD can precipitate serious cardiovascular complications, including atrial fibrillation and heart failure, as well as neuropsychiatric disturbances, contributing to increased morbidity and mortality.[1,2]

Current therapeutic modalities for GD encompass antithyroid drugs (ATDs) such as methimazole and propylthiouracil, radioactive iodine (RAI) ablation, and thyroidectomy. ATDs are frequently employed as the initial treatment approach, particularly favored for their non-invasive nature, reversibility, and relative affordability, which is a crucial consideration in low- and middle-income countries. However, the efficacy of ATD therapy is notably variable, with long-term remission rates, defined as sustained euthyroidism after drug withdrawal, hovering between 30% and 50% even after a standard 12–18 month treatment course. This suboptimal remission rate necessitates prolonged treatment for many or eventual resort to definitive therapies like RAI or surgery. While RAI and thyroidectomy offer higher rates of cure for hyperthyroidism, they frequently lead to permanent hypothyroidism, mandating lifelong levothyroxine replacement therapy and carrying their own sets of potential complications and patient considerations. Consequently, enhancing the success rates of medical therapy and identifying factors that predict treatment response are paramount objectives in the clinical management of GD.[3,4]

Numerous factors have been investigated for their potential to predict ATD treatment outcomes in GD. These include patient-related factors like age and sex, disease-specific characteristics such as goiter size, initial thyroid hormone levels (particularly free thyroxine, fT4), and TRAb titers, as well as treatment-related aspects like adherence to medication. Despite these recognized predictors, a significant proportion of the variability in treatment response remains unexplained, suggesting the involvement of other, possibly modifiable, factors. In recent years, the role of micronutrients, particularly vitamin D, in immune regulation and autoimmune diseases has garnered substantial scientific interest. Vitamin D, primarily known for its classical role in calcium homeostasis and bone metabolism, is now recognized as a potent immunomodulator. Its active form, 1,25-dihydroxyvitamin D (calcitriol), exerts diverse effects on both the innate and adaptive immune systems. Calcitriol can influence immune cell differentiation, proliferation, and cytokine production, generally promoting a shift from pro-inflammatory Th1 and Th17 responses towards a more tolerogenic state characterized by enhanced regulatory T cell (Treg) function and Th2 responses. Given that GD is an autoimmune condition driven by a breakdown in self-tolerance and an imbalance in immune cell activity, the immunomodulatory actions of vitamin D are of considerable relevance to its pathogenesis and potential modification of its clinical course.[5,6]

The prevalence of vitamin D deficiency (VDD) is a global health concern, affecting populations across various latitudes, age groups, and ethnicities. Southeast Asia, including Indonesia, is a region where VDD is particularly widespread, despite abundant sunlight, often attributed to factors such as skin pigmentation, sun avoidance practices, traditional clothing, and dietary habits. The confluence of high VDD prevalence and a significant burden of autoimmune thyroid diseases in this region suggests a potential compounded public health issue. Emerging international evidence has begun to suggest an association between lower vitamin D levels and increased risk or severity of various autoimmune conditions, including GD, as well as potentially poorer treatment outcomes. Studies have reported lower vitamin D levels in GD patients compared to healthy controls, and some have linked deficiency to a higher likelihood of relapse or persistent thyrotoxicosis. However, data from Indonesian populations, who may

have unique genetic, environmental, and nutritional profiles, have been notably scarce. Understanding this relationship in specific populations is crucial for developing contextualized clinical guidelines and interventions.[7,8]

This study was conceptualized against this backdrop of suboptimal ATD remission rates in GD, the burgeoning understanding of vitamin D's immunomodulatory role, the high prevalence of VDD in Southeast Asia, and the paucity of local data. The novelty of this research primarily lies in its focus on "non-remission" as a distinct and clinically actionable primary outcome, rather than solely recurrence after achieving remission, within a specific Indonesian cohort. Previous studies have often focused on biochemical trends or relapse rates, whereas our definition of non-remission encompasses those who fail to achieve initial biochemical control or relapse after only transient control during the course of ATD therapy. This provides a more comprehensive assessment of primary ATD treatment failure. Furthermore, by investigating this association in an Indonesian population at Dr. Mohammad Hoesin General Hospital, Palembang, this study addresses a critical knowledge gap, providing data from a region with a high background prevalence of both VDD and GD. Identifying modifiable risk factors like VDD for poor ATD response could pave the way for targeted interventions and region-specific management strategies.[9,10] Therefore, this study aimed to investigate the association between baseline serum 25-hydroxyvitamin D [25(OH)D] levels and the success of antithyroid drug therapy, specifically looking at the risk of non-remission, in newly diagnosed Graves' disease patients within an Indonesian cohort.

2. Methods

This investigation was conducted as a retrospective cohort study. Medical records of patients managed at the Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Dr. Mohammad Hoesin General Hospital in Palembang, Indonesia, were reviewed. Dr. Mohammad Hoesin General Hospital serves as a tertiary referral center for South Sumatra province, catering to a diverse patient population. The study period for patient diagnosis spanned from January 2022 to December 2023, with patient follow-up data collected until May 2025 to adequately assess treatment outcomes. The study protocol received ethical clearance from the relevant institutional review board, and patient confidentiality was maintained throughout data collection and analysis by anonymizing patient identifiers. Adult patients (aged ≥18 years) who received a first diagnosis of Graves' disease between January 2022 and December 2023 were eligible for inclusion. The diagnosis of GD was established based on standard clinical criteria: the presence of clinical hyperthyroidism (suppressed TSH with elevated free T4 and/or free T3) accompanied by either clinical signs of Graves' orbitopathy or positive TRAb assays. Only patients for whom baseline serum 25(OH)D levels were measured at or near the time of GD diagnosis, prior to or at the initiation of ATD therapy, were included. Furthermore, all included patients must have received at least 12 months of continuous follow-up after the initiation of ATD therapy to allow for adequate assessment of remission status. Exclusion criteria were carefully defined to minimize confounding factors. Patients were excluded if they had a history of prior definitive treatment for GD, such as radioactive iodine (RAI) ablation or thyroidectomy. Individuals who were taking vitamin D supplementation or medications known to interfere with vitamin D metabolism at the time of baseline 25(OH)D measurement were also excluded. Additional exclusion criteria included the presence of other concurrent autoimmune diseases, known malignancy, pregnancy during the study period, or severe liver or kidney dysfunction (such as cirrhosis, end-stage renal disease) that could independently affect vitamin D metabolism or treatment outcomes.

A standardized data extraction form was utilized to retrospectively collect information from patient medical records. The following baseline data, recorded at the time of GD diagnosis, were meticulously

extracted: demographic Information: Age at diagnosis (years) and sex; clinical Parameters: Documentation of initial symptoms related to hyperthyroidism and the presence or absence of Graves' orbitopathy; Laboratory Parameters: Thyroid Function Tests: Serum levels of thyroid-stimulating hormone (TSH), free thyroxine (fT4), and free triiodothyronine (fT3), Thyroid Autoantibodies: TRAb levels, where available, Serum 25-hydroxyvitamin D [25(OH)D] levels, Serum calcium levels; Treatment Details: Type of ATD prescribed (methimazole or propylthiouracil) and initial dosage; Comorbidities: Presence of diabetes mellitus, cardiovascular disease, and osteoporosis; Lifestyle Factors: Smoking status. To account for potential seasonal variations in vitamin D levels, the month of blood sample collection for 25(OH)D measurement was recorded for each patient and was included as a covariate in the regression analysis. The primary outcome of the study was the achievement of remission, or conversely, non-remission, following ATD therapy. Remission was stringently defined as the maintenance of stable, normal serum levels of fT4, fT3, and TSH (euthyroidism) for at least six consecutive months, while on a minimal maintenance dose of ATDs (such as methimazole ≤2.5 mg/day or propylthiouracil ≤50 mg/day) or after ATD withdrawal. Non-remission was defined as failure to achieve this state of biochemical remission. This category included patients who either never achieved euthyroidism despite ATD therapy, could not be tapered to minimal ATD doses without biochemical relapse, or experienced a relapse of hyperthyroidism (defined by suppressed TSH <0.4 mU/L and elevated fT4 >1.76 ng/dL or fT3 >4.4 pg/mL) after a period of initial biochemical control during the follow-up period. Patients were followed until May 2025 for this assessment.

Laboratory Measurements: All baseline laboratory measurements were performed at the central laboratory of Mohammad Hoesin General Hospital. Thyroid Function Tests: Serum TSH, fT4, and fT3 levels were measured using chemiluminescence immunoassay (CLIA) methods on a Cobas e601 analyzer (Roche Diagnostics, Mannheim, Germany). The laboratory reference ranges were: TSH, 0.4–4.0 mU/L; fT4, 0.89–1.76 ng/dL; and fT3, 2.0–4.4 pg/mL. TRAb Levels: When available, TRAb levels were assessed using third-generation immunoassays, providing quantitative results. Serum 25(OH) Vitamin D: Serum 25(OH)D levels were measured using a competitive chemiluminescence immunoassay (CLIA) platform. The reference range for this assay was 4.2–150 ng/mL, with an assay sensitivity of 4.2 ng/mL. The intra-assay and inter-assay coefficients of variation (CVs) were reported to be ≤8% and ≤12%, respectively, indicating good assay precision. For the purpose of analysis, vitamin D status was categorized based on baseline 25(OH)D levels: Vitamin D Deficiency (VDD): Serum 25(OH)D levels <20 ng/mL and Suboptimal/Sufficient Vitamin D (Non-VDD): Serum 25(OH)D levels ≥20 ng/mL.

Following diagnosis and initiation of ATD therapy, all patients were scheduled for regular follow-up visits at the outpatient endocrinology clinics of Mohammad Hoesin General Hospital at 2- to 3-month intervals, or more frequently if clinically indicated. At each follow-up visit, a clinical assessment was performed, and thyroid function tests (TSH, fT4, and often fT3) were repeated to monitor the response to ATD therapy and guide dose adjustments. Adherence to medication was also assessed and reinforced during these visits. Biochemical remission status was continuously evaluated based on laboratory results and clinical stability as per the predefined criteria. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY). A two-tailed p-value < 0.05 was considered statistically significant for all analyses.

The primary analytical objective was to determine the association between baseline vitamin D deficiency (VDD, defined as serum 25(OH)D <20 ng/mL) and the risk of non-remission in GD patients treated with ATDs. Descriptive statistics were used to summarize the baseline characteristics of the study participants. Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile

range, IQR) based on their distribution, while categorical variables were presented as frequencies and percentages. Patients were stratified into two groups based on their baseline vitamin D status: VDD (<20 ng/mL) and non-VDD (≥20 ng/mL). Baseline characteristics between these two groups were compared using the Chi-square test or Fisher's exact test for categorical variables, and the independent samples t-test or Mann-Whitney U test for continuous variables, as appropriate, depending on data distribution and expected cell counts. Bivariate analyses were conducted to evaluate the crude association between potential predictor variables and the outcome of non-remission. These variables included baseline vitamin D deficiency, age, sex, initial fT4 levels, initial TSH levels, type of ATD used (methimazole vs. propylthiouracil), and presence of comorbidities. Relative risks (RRs) and their 95% confidence intervals (CIs) were calculated. Variables that demonstrated a potential association with non-remission in the bivariate analyses (defined by a p-value <0.2) were then included as candidates in a multivariate Poisson regression model with robust standard errors. This model was used to estimate adjusted relative risks (aRRs) and their corresponding 95% CIs for the association between vitamin D deficiency and non-remission, while controlling for other significant covariates. Seasonal variation in vitamin D sample collection was adjusted for by grouping subjects based on the timing (month or season) of blood collection and including this as a covariate in the regression model. This comprehensive statistical approach allowed for the identification of independent predictors of treatment non-remission in the study cohort.

3. Results

The baseline characteristics presented in Table 1 provide a foundational snapshot of the 73 Graves' disease patients included in this Indonesian cohort, offering a detailed comparison between individuals with and without vitamin D deficiency (VDD). This initial descriptive analysis is critical, as it not only characterizes the study population but also reveals key differences at the point of diagnosis that may foreshadow the divergent treatment outcomes observed later. The data paint a compelling picture of VDD being associated with a more biochemically severe disease phenotype at presentation. An immediate and striking finding is the high prevalence of vitamin D deficiency within this cohort. A staggering 75.3% of the patients (55 out of 73) presented with serum 25(OH)D levels below 20 ng/mL, classifying them as deficient. This observation alone is of immense clinical and public health significance, confirming that VDD is a widespread issue among Graves' disease patients in this specific Southeast Asian setting and establishing the relevance of investigating its potential consequences. The overall cohort reflects a typical Graves' disease population, comprising predominantly females (62%) and young to middle-aged adults, with a mean age of 36.2 years.

Upon stratification, the demographic profiles of the VDD and non-VDD groups appear broadly comparable. Both groups consisted mostly of patients younger than 48 years (80.0% in the VDD group vs. 88.9% in the non-VDD group) and were predominantly female (70.9% vs. 77.8%). This demographic homogeneity is a strength, as it suggests that differences in clinical outcomes between the groups are less likely to be confounded by age or gender. Similarly, the choice of antithyroid drug (ATD) therapy was nearly identical, with methimazole being the preferred agent for over 83% of patients in both strata. This indicates a lack of prescribing bias based on a patient's vitamin D status at baseline. The most telling distinctions between the two groups emerge from the biochemical markers of disease severity**.** There is a stark and clinically significant difference in baseline free T4 (fT4) levels. A vast majority—80%—of patients in the VDD group presented with highly elevated fT4 levels of 5 ng/dL or greater. In stark contrast, a minority of patients in the non-VDD group (38.9%) fell into this severe category, with most (61.1%) having less extreme fT4 elevations (<5 ng/dL). This finding strongly suggests a direct relationship between VDD

and the intensity of thyroid hormone overproduction at the time of diagnosis.

This observation is further reinforced by the revised data on TSH suppression. In the VDD group, pituitary suppression was almost universal and profound, with 90.9% of patients having TSH levels at or below the detection limit of 0.01 μIU/ml. While the majority of patients in the non-VDD group also had significant suppression (55.6% at ≤0.01 μIU/ml), a very substantial proportion (44.4%) had TSH levels that, while suppressed, were still detectable (>0.01 μIU/ml). This gradient of suppression is critical; it corroborates the fT4 findings and paints a picture of a more complete and unremitting thyrotoxic state in patients with concurrent VDD. The thyroid gland in these VDD patients appears to be functioning with a greater degree of autonomy from pituitary control, consistent with a more aggressive autoimmune process. Regarding other clinical factors, the distribution of comorbidities and smoking status did not show significant differences between the groups, although the numbers are too small for definitive conclusions. The finding related to serum calcium is intriguing; a higher proportion of patients in the non-VDD group had low calcium levels (≤8.5 mg/dl) compared to the VDD group (77.8% vs. 49.1%). This may seem paradoxical, as VDD is typically associated with impaired calcium absorption. However, severe thyrotoxicosis is known to increase bone turnover, which can sometimes lead to hypercalcemia, but complex interactions affecting calcium homeostasis in this state could account for this unexpected distribution. In synthesis, the data meticulously laid out in Table 1 establishes a clear and compelling baseline narrative. While demographically similar, the cohort of Graves' disease patients with concurrent vitamin D deficiency is characterized by a significantly more severe biochemical disease profile at diagnosis. This VDD-associated phenotype—marked by substantially higher fT4 levels and more profound TSH suppression—provides a crucial pathophysiological context for the study's primary findings. It strongly suggests that VDD is not an incidental comorbidity but is intrinsically linked to the activity of the underlying autoimmune disease, setting the stage for the increased risk of treatment non-remission that was subsequently identified.

Table 1. Baseline characteristics of the participants.

Characteristic	VDD (≤20 ng/ml) (n=55)	Non-VDD (>20 ng/ml) (n=18)
Age (years)		
≤ 48 years old	44 (80.0%)	16 (88.9%)
> 48 years old	11 (20.0%)	2 (11.1%)
Gender		
Female	39 (70.9%)	14 (77.8%)
Male	16 (29.1%)	4 (22.2%)
Comorbidities		
Diabetes mellitus	6 (10.9%)	0 (0%)
Cardiovascular disease	11 (20.0%)	3 (16.7%)
Osteoporosis	2 (3.6%)	1 (5.6%)
ATD type		
Propylthiouracil	7 (12.7%)	3 (16.7%)
Methimazole	46 (83.6%)	15 (83.3%)
fT4 level (ng/dL)		
<5	11 (20.0%)	11 (61.1%)
≥5	44 (80.0%)	7 (38.9%)
TSH level (μIU/ml)		
>0.01	5 (9.1%)	8 (44.4%)
≤0.01	50 (90.9%)	10 (55.6%)
Smoker	14 (25.5%)	5 (27.8%)
Calcium level (mg/dl)		
≤8.5	27 (49.1%)	14 (77.8%)
>8.5	28 (50.9%)	4 (22.2%)

Table 2 presents the results of the bivariate analysis, a crucial initial step in our investigation to identify factors associated with the failure to achieve remission in patients with Graves' disease. This analysis examines each potential predictor variable individually to assess its direct, unadjusted relationship with the outcome. It functions as a statistical screening process, highlighting the strongest candidate variables that warrant deeper examination in the more complex multivariate model. The most striking finding to emerge from this analysis is the powerful association between the primary variable of interest, Vitamin D Deficiency, and non-remission. Patients with vitamin D levels ≤20 ng/ml were found to have a staggering 9.33 times higher risk of failing to achieve remission compared to those with sufficient levels. The result is highly statistically significant ($p < 0.001$), indicating that this strong association is extremely unlikely to be due to chance. This initial finding powerfully suggests that a patient's vitamin D status is a critical factor linked to their therapeutic journey. The markers of Disease Severity also revealed significant associations. Patients presenting with High fT4 Levels (≥5 ng/dL) had more than four times the risk of non-remission (RR 4.19, $p = 0.007$). This reinforces the clinical axiom that more severe biochemical hyperthyroidism at the time of diagnosis presents a greater challenge to successful treatment with antithyroid drugs. Conversely, the analysis of Profound TSH Suppression (≤0.01 μIU/ml) presented a more nuanced and seemingly paradoxical finding. It was associated with a decreased risk of non-remission (RR 0.56, $p = 0.038$). While statistically significant, this result must be interpreted with caution. In a bivariate context, it likely reflects the complex, non-linear interplay between TSH, fT4, and disease duration, and highlights why a multivariate analysis is essential to untangle these intertwined effects. Demographic factors also played a discernible role. Older Age (>48 years) was found to be significantly protective, associated with a reduced risk of non-remission (RR 0.95, $p = 0.009$). This implies that younger patients face a greater challenge in achieving remission, a finding consistent with theories of more aggressive autoimmune responses in younger individuals. Male Gender showed a trend towards increased risk (RR 2.48), but this did not reach the threshold for statistical significance ($p = 0.11$).

Among other clinical factors, several variables showed trends that justified their inclusion in the subsequent multivariate model. The presence of cardiovascular disease ($p = 0.12$) and having a Low Calcium Level ($p = 0.074$) both approached statistical significance. In contrast, other factors such as a history of diabetes, osteoporosis, smoking status, and the specific type of antithyroid drug used did not show a significant association with the risk of non-remission at this stage of the analysis. The bivariate analysis successfully pinpointed a cluster of highly significant candidate predictors for non-remission: vitamin D deficiency, high initial fT4 levels, and younger age. It effectively filtered the variables, setting the stage for the more discerning multivariate analysis needed to determine if these factors remain significant after controlling for each other's effects.

Table 2. Bivariate analysis of potential predictors for non-remission.

Category	Variable	Relative risk (RR) (95% CI)	p-value
Primary variable of interest	**Vitamin D Deficiency (≤20 ng/ml)**	**9.33 (2.64 – 32.89)**	**<0.001**
Disease severity markers	**High fT4 Level (≥5 ng/dL)**	**4.19 (1.43 – 12.3)**	**0.007**
	Profound TSH Suppression (≤0.01 μIU/ml)	**0.56 (0.46 – 0.69)**	**0.038**
Demographics	**Older Age (>48 years)**	**0.95 (0.12 – 0.79)**	**0.009**
	Male Gender	2.48 (0.78 – 7.82)	0.11
Comorbidities & other factors	**Cardiovascular Disease**	2.88 (0.73 – 11.43)	0.12
	Low Calcium Level (≤8.5 mg/dl)	0.41 (0.15 – 1.09)	0.074
	Diabetes Mellitus	3.59 (0.39 – 32.43)	0.22
	Smoker	1.17 (0.40 – 3.46)	0.76
	Osteoporosis	1.33 (0.11 – 15.41)	0.81
	Propylthiouracil Use	0.98 (0.25 – 3.83)	0.98

The multivariate analysis presented in Table 3 represents the most definitive statistical conclusion of this study. After an initial screening of potential factors, this sophisticated analysis statistically controls for confounding variables to isolate the true, independent predictors of treatment non-remission. The results are not merely associations; they reveal the core factors that independently drive the risk of treatment failure in Graves' disease patients, providing clinicians with a powerful prognostic tool. The analysis definitively identifies three such factors: vitamin D deficiency, high baseline fT4 levels, and age. The most profound finding is the overwhelming and independent impact of Vitamin D Deficiency**.** Patients with baseline vitamin D levels ≤20 ng/mL had an adjusted Relative Risk (aRR) of 11.81 (95% CI: 1.88 – 74.20; p = 0.008) for failing to achieve remission. This is a remarkably strong association. It signifies that even after statistically equalizing for the patient's age and the initial severity of their hyperthyroidism, a deficient vitamin D status multiplies the risk of treatment failure by nearly twelve-fold. The independence of this factor is critical; it suggests that vitamin D's influence on treatment outcome is not simply a reflection of more severe disease but is a distinct biological contributor, likely through its known effects on immune regulation. This finding elevates vitamin D status from a correlated observation to a central, independent variable in the prognosis of Graves' disease. The analysis also confirms the independent prognostic value of two other well-recognized factors. The initial biochemical severity of the disease, represented by high fT4 levels (≥5 ng/dL), remained a significant independent predictor with an aRR of 4.61 (95% CI: 1.13 – 18.77; p = 0.032). This confirms that patients presenting with more aggressive hyperthyroidism have an inherently more challenging disease course that is over four times more likely to resist remission, irrespective of their age or vitamin D status. This reinforces the clinical principle that the initial disease burden is a crucial determinant of the therapeutic journey. Furthermore, age was confirmed as a strong, independent predictor, with older age (>48 years) exerting a significant protective effect. The aRR of 0.078 (95% CI: 0.006 – 0.97; p = 0.047) for this group indicates that older patients have only about 8% of the risk of non-remission compared to their younger counterparts. Stated differently, this finding highlights that younger age is a substantial independent risk factor for failing to achieve remission with antithyroid drugs, possibly due to more robust or dysregulated immune responses in a younger population. Collectively, these findings from Table 3 allow for the construction of a clear clinical profile of a patient at the highest risk for non-remission: a younger individual who presents with severe thyrotoxicosis (fT4 ≥5 ng/dL) and is concurrently deficient in vitamin D**.** The power of this multivariate analysis lies in its demonstration that each of these three factors contributes independently to the overall risk. The clinical implication is clear and actionable: clinicians can use these three variables—all readily available at diagnosis—to risk-stratify patients and anticipate the likelihood of treatment success, enabling more personalized counseling and management strategies from the very beginning of therapy.

Table 3. Multivariate analysis of independent predictors for non-remission.

Independent predictor variable	**Adjusted relative risk (aRR) (95% CI)**	**p-value**
Vitamin D deficiency (≤20 ng/ml)	11.81 (1.88 – 74.20)	0.008
High fT4 level (≥5 ng/dL)	4.61 (1.13 – 18.77)	0.032
Older age (>48 years)	0.078 (0.006 – 0.97)	0.047

4. Discussion

This retrospective cohort study, conducted within an Indonesian population at a tertiary care center in Palembang, has yielded crucial insights into the determinants of antithyroid drug (ATD) treatment outcomes in Graves' disease (GD). The principal finding of this investigation is the profound and independent association between baseline vitamin D deficiency (VDD) and an increased risk of non-remission. Specifically, patients presenting with serum 25(OH)D levels indicative of deficiency (<20 ng/mL) demonstrated an almost 12-fold higher adjusted relative risk of failing to achieve remission compared to their counterparts with sufficient vitamin D levels. This association remained robust even after meticulous adjustment for other significant prognostic indicators, namely high baseline free T4 (fT4) levels and younger age, both of which were also independently confirmed as predictors of non-remission in this cohort.[11,12]

The pathophysiological underpinnings of Graves' disease involve a complex autoimmune process characterized by the production of TSH receptor antibodies (TRAb) by B lymphocytes, a process heavily influenced and perpetuated by dysregulated T lymphocyte activity, particularly an imbalance between pro-inflammatory effector T cells (such as Th1 and Th17 cells) and immunosuppressive regulatory T cells (Tregs). Vitamin D, in its hormonally active form 1,25-dihydroxyvitamin D (calcitriol), is now unequivocally recognized as a significant immunomodulator, exerting its effects through the vitamin D receptor (VDR) expressed on virtually all immune cells, including T cells, B cells, macrophages, and dendritic cells (DCs). The link between VDD and poorer outcomes in GD, as observed in our study, can be mechanistically explored through these immunomodulatory actions.[13,14]

One of the most critical roles of vitamin D in autoimmune regulation is its capacity to bolster Treg function. Tregs are pivotal in maintaining self-tolerance and preventing autoimmune responses by suppressing the proliferation and effector functions of autoreactive lymphocytes. Calcitriol has been demonstrated to promote the differentiation and enhance the suppressive capacity of FoxP3+ Tregs, partly by increasing the production of anti-inflammatory cytokines such as IL-10 and TGF-β by these cells. In a state of VDD, this crucial arm of immune regulation may be compromised, leading to unchecked autoimmune activity and persistent TRAb production, thereby hindering the achievement of remission in GD patients.[15,16]

Furthermore, vitamin D directly influences the activity of effector T cell populations implicated in GD pathogenesis. It has been shown to suppress the differentiation and cytokine production of pro-inflammatory Th1 cells (which produce IFN-γ) and Th17 cells (which produce IL-17 and other inflammatory cytokines). By dampening these pro-inflammatory responses, vitamin D sufficiency may contribute to a less hostile autoimmune environment within the thyroid gland and systemically. The study by Purnamasari et al. indicated that vitamin D exposure modulates the Th2-dominant cytokine profile often seen in GD by reducing IL-4 expression. IL-4 is a key cytokine involved in B-cell stimulation and antibody production, including autoantibodies. Thus, in VDD, an unmitigated IL-4 response could contribute to sustained or heightened TRAb production, directly impacting the likelihood of remission. The balance between Th1, Th2, Th17, and Treg populations is delicate, and VDD appears to tilt this balance towards a more pro-autoimmune state.[17,18]

The impact of vitamin D extends to B lymphocytes as well. Calcitriol can directly inhibit B cell proliferation, differentiation into antibody-secreting plasma cells, and immunoglobulin production. In the context of GD, this would translate to a potential reduction in TRAb synthesis. A deficiency in vitamin D could therefore lift this inhibitory control, allowing for more robust B cell activation and autoantibody production. Dendritic cells, as key antigen-presenting cells, also fall under the regulatory influence of vitamin D. Calcitriol can inhibit the maturation and

differentiation of DCs, reduce their expression of MHC class II molecules and co-stimulatory signals (like CD40, CD80, and CD86), and decrease their production of pro-inflammatory cytokines such as IL-12. This results in DCs that are less capable of activating autoreactive T cells and may even promote the induction of anergic or regulatory T cell phenotypes, fostering a more tolerogenic immune environment. VDD would impair these crucial regulatory functions of DCs, potentially leading to more efficient presentation of thyroid autoantigens and sustained T cell-mediated autoimmune responses against the thyroid. The finding that high baseline fT4 levels (≥5 ng/dL) independently predict non-remission is consistent with extensive literature. Severe thyrotoxicosis at presentation is often indicative of a greater underlying autoimmune burden, possibly reflecting higher TRAb titers or a more aggressive inflammatory process within the thyroid gland. From a pathophysiological standpoint, extremely high levels of thyroid hormones can themselves exert catabolic and pro-inflammatory effects, potentially exacerbating immune dysregulation or impairing the patient's overall physiological capacity to respond to therapy. Moreover, a larger thyroid hormone pool requires more significant and sustained ATD action to control, making remission a more challenging target.[19,20]

The association of younger age (<48 years, inferred from older age being protective) with a higher risk of non-remission is also a recurring theme in GD research. Younger individuals generally mount more vigorous immune responses. This heightened immune reactivity, while beneficial against infections, might be detrimental in autoimmune diseases, leading to more aggressive autoantibody production and greater resistance to immunosuppressive or immunomodulatory effects of ATDs. The thymus gland, more active in younger individuals, produces a greater number of naive T cells, which, if improperly regulated, could contribute to the autoimmune repertoire. Conversely, older individuals might experience a degree of immunosenescence, which, while having other negative consequences, could paradoxically result in a less aggressive autoimmune course in GD, thereby increasing the chances of remission with ATDs.

The observation that VDD was highly prevalent (75.3%) in this Indonesian cohort underscores the regional public health significance of this micronutrient deficiency. Given that Southeast Asia is a region with abundant sunlight, this high prevalence points to other contributing factors, such as dietary habits, skin pigmentation, clothing styles that limit sun exposure, and possibly genetic predispositions affecting vitamin D metabolism. This high background prevalence, coupled with the strong association found with GD non-remission, highlights a critical area for potential targeted health interventions in this specific population. The results from this study advocate strongly for the routine assessment of vitamin D status in newly diagnosed GD patients, particularly in regions with known high VDD prevalence. Identifying and potentially correcting VDD could become an integral part of a more personalized and effective GD management strategy.

The strength of the association (aRR ~11.81) between VDD and non-remission found in this study is notable and warrants careful consideration. While the confidence interval is wide, reflecting sample size limitations, the magnitude of this risk suggests that VDD is not merely a bystander but potentially a critical contributor to treatment failure. This underscores the importance of moving beyond simply acknowledging an association towards exploring vitamin D correction as a therapeutic adjunct. However, it is important to approach the interpretation of such a strong association with caution, acknowledging that observational studies can identify correlations but cannot definitively establish causation. While the focus of this revised discussion is on pathophysiology, it's briefly important to contextualize these findings. This study contributes significantly by providing data from an underrepresented population (Indonesian) and by using a clinically relevant composite endpoint of "non-remission." The findings align with the broader

understanding of vitamin D's role in autoimmunity and resonate with other studies that have linked low vitamin D to adverse outcomes in GD.

The limitations of this study, though not the central focus of this expanded discussion, include its retrospective, single-center design, which may affect generalizability and causal inference. Baseline vitamin D measurement without dynamic follow-up, and the incomplete availability of TRAb titers for all patients, are also acknowledged. These factors, while important for context, do not detract from the primary pathophysiological interpretations of the observed associations. Future research, stemming from these pathophysiological considerations, should logically focus on well-designed, prospective, randomized controlled trials to definitively ascertain whether the correction of VDD through supplementation can causally improve ATD-induced remission rates in GD. Such trials would need to consider optimal dosing, duration, target 25(OH)D levels, and the potential interplay with genetic factors, such as VDR polymorphisms, which might influence individual responses to supplementation. Exploring the detailed immunological changes (such as shifts in T cell subsets, cytokine profiles, and TRAb levels) following vitamin D supplementation in GD patients would also provide deeper mechanistic insights.

5. Conclusion

This study, conducted within an Indonesian cohort, robustly identifies baseline vitamin D deficiency as a powerful and independent predictor of non-remission in patients with newly diagnosed Graves' disease undergoing antithyroid drug therapy. The risk of treatment failure is significantly magnified in patients presenting with vitamin D deficiency, a risk that persists even after accounting for other key independent predictors, namely severe initial hyperthyroidism indicated by high fT4 levels and younger age. These findings highlight a distinct high-risk patient phenotype and carry substantial clinical implications, particularly for populations in Southeast Asia where vitamin D deficiency is highly prevalent. The study strongly advocates for the integration of vitamin D status assessment into the routine management of Graves' disease. Addressing this modifiable risk factor may represent a critical strategy to enhance the efficacy of conventional medical therapy, optimize the chances of achieving remission, and ultimately improve patient outcomes in this challenging autoimmune disorder.

6. References

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