https://doi.org/10.29309/TPMJ/2026.33.05.10217 www.theprofesional.com

ORIGINAL ARTICLE

Interplay between vitamin D, Thyroid function tests and Thyroid Peroxidase antibodies in female patients with sub-clinical hypothyroidism reporting to a Tertiary Care Hospital in Rawalpindi, Pakistan.

Wafa Omer[1], Omer Jamshed Khan[2], Riaz Shahbaz Janjua[3], Muhammad Umar[4], Saira Karimi[5]

ABSTRACT… Objective: To assess the correlation between Serum Vitamin D levels, thyroid function tests and Thyroxine Peroxidase Antibosies (TPOAb) levels in the female patients of sub-clinical hypothyroidism. **Study Design:** Case Control study. **Setting:** HBS General Hospital and Department of Chemical Pathology, HBS Medical and Dental College, Islamabad. **Period:** Jun 2023 to Dec 2023. **Methods:** Total of 790 female subjects aged 18 to 45 years reporting to the female Endocrine Clinic were consecutively recruited, out of which 450 participated in the study. Those with serum TSH >4.3 to 10 mU/l and normal levels of fT4 and fT3 and TPOAb positive were recruited as cases (n=200). Those who had normal TSH between 0.4 to 4.0 mIU/L and TPOAb negative were recruited as controls (n=250). **Results:** BMI and smoking was higher amongst controls. In biochemical parameters, there was a highly significant negative correlation between the serum TSH levels and TPO antibodies with the serum 25 (OH) Vit-D level (p<0.0001).fT4 showed a moderately significant positive correlation with serum 25 (OH) Vit-D level (p<0.001) while fT3 did not show any significant correlation with the serum 25 (OH) Vit-D levels. **Conclusion:** The identified correlations emphasize the importance of comprehensive thyroid assessments, including vitamin D levels, in the clinical management of sub-clinical hypothyroidism.

Key words: Correlation, Sub-clinical Hypothyroidism, Thyroid Function Tests, Thyroid Peroxidase Antibodies, Vitamin-D Levels.

Article Citation: Omer W, Khan OJ, Janjua RS, Umar M, Karimi S. Interplay between vitamin D, Thyroid function tests and Thyroid Peroxidase antibodies in female patients with sub-clinical hypothyroidism reporting to a Tertiary Care Hospital in Rawalpindi, Pakistan. Professional Med J 2026; 33(05):840-845. https://doi.org/10.29309/TPMJ/2026.33.05.10217

INTRODUCTION

Sub-clinical hypothyroidism is associated with elevated serum levels of thyroid-stimulating hormone (TSH); however, the serum levels of thyroxine (fT4) and triiodothyronine (fT3) remain normal. According to the reported data, the levels of TSH ranging between 4.3-10 mIU/L are found to be linked with subclinical hypothyroidism.[1] As per the reports of developed countries, the burden of sub-clinical hypothyroidism is 4 to 15 percent.[2] Whereas the literature review from NHANES III surveys and the Whickham study from the UK reported the prevalence from 4.3% and 7.5% respectively.[3] However, in Pakistan, the prevalence of hypothyroidism is 4.1% in adults and 5.4% in children.[4] Moreover, a gender-based study in Northern Pakistan indicated the higher prevalence of sub-clinical hypothyroidism in females.[5]

Sub-clinical hypothyroidism is also termed as mild thyroid failure.[1] Patients of sub-clinical hypothyroidism present with a wide array of non-specific symptoms; however, the risk of dyslipidemias, cardiovascular disorders, diabetes, and fetal anomalies remains high in females suffering from this disease.[6] An association has been observed between low serum vitamin D levels and the presence of hypothyroidism.[3] Another study showed inverse correlation between 25 (OH) Vitamin D3 and TSH in women of reproductive age.[7] However, a study carried out in Northern India shows no association between vitamin D levels and thyroid-stimulating hormone.[8] The association between the vitamin D levels, Thyroid function tests, and Thyroid Peroxidase anti-bodies (TPOAb) in subclinical hypothyroidism remains controversial, especially in the Pakistani population. Therefore, the current study was directed towards evaluating the serum levels of vitamin D, TSH, fT4, fT3, and TPOAb in cases and controls to expound the association

1. MBBS, Ph.D, Professor Pathology, RMU.
2. MBBS, FCPS, Associate Professor Rehabilitation Medicine, AFIRM.
3. MBBS, FCPS, Professor Medicine, HBS Medical College,
4. MBBS, FCPS, Professor Medicine, RMU.
5. M.Phil, PhD, Research Officer, BUHSCI.

Correspondence Address:
Saira Karimi
BUHSCI.
sairakarimi.buhsci@bahria.edu.pk

Article received on:
04/11/2025
Accepted for publication:
13/01/2026

between vitamin D serum levels and thyroid functional and immunological parameters in females with subclinical hypothyroidism. The current study analysis indicated a significant negative correlation between 25-hydroxyvitamin D levels and TSH and TPOAb concentrations (p< 0.0001). Conversely, a moderate positive correlation was found between serum 25-hydroxyvitamin D levels and fT4 levels (p<0.001). However, a statistically insignificant relationship was observed between serum vitamin D levels and fT3.

METHODS

Participants for the case-control association study were recruited from the HBS Medical and Dental College (Chemical Pathology Department) in Islamabad and HBS General Hospital in Islamabad. The current study conforms to the Helsinki Declaration and was also approved by the institutional Ethics Review Board over a period of six months (June to December 2023), bearing number HBS/IRB/18/186 dated 17 April 2023.

Inclusion Criteria

1. Only the female gender
2. Ages 18-45 years
3. Participants with normal serum concentrations of fT4 and fT3 and having mildly elevated TSH levels (greater than 4.3 and up to 10 mU/L). The samples with TPOAb positive were taken as cases (n=200).
4. Participants with normal TSH between 0.4 and 4.0 mIU/L and TPOAb negative were recruited as controls (n=250).

Exclusion Criteria

1. Male Gender
2. Subjects with infectious or other autoimmune diseases, bone disorders, carcinomas, diabetes, familial hyperlipidemia, cardio-vascular heart disease, rheumatoid arthritis, and pregnant females.
3. Those taking levothyroxine medication or vitamin D supplements.
4. Among the control participants, individuals with acute illnesses, those taking vitamin supplements, and those who did not provide consent were excluded.

Detailed demographic and clinical data were collected and validated by the collaborating trained general practitioner following informed consent. Among the participants, those with serum TSH greater than 4.3 and up to 10 mU/l and having normal ranges of fT4 and fT3 levels[1] with TPOAb positive were grouped as cases (n=200). Participants with normal TSH between 0.4 and 4.0 mIU/L and TPOAb negative were grouped as controls (n=250). Blood samples (7ml) were collected each morning between 8:00 and 11:30 AM by venepuncture and were transferred to a plain vacutainer tube for serum separation and analysis. Serum was separated by centrifugation at 1500x g for 15 minutes and stored at -70°C until biochemical analysis. Serum 25(OH) Vit-D was estimated using the chemiluminescence method on the ADVIA Centaur system, while serum TSH,fT4,fT3, and TPOAb were estimated using the chemiluminescent immunoassay method on Access 2 hormone Analyzer (Beckman Coulter US). Vitamin-D levels were categorised as (a) Vitamin-D deficiency 12-25nmol/L, (b) Vitamin-D insufficiency 25-50nmol/L, (c) Marginal Vitamin-D status 50 to 75nmol/L and (d) Vitamin-D sufficiency 75-150nmol/L (Appunni et al.,2021). The data were analysed using SPSS software v21 (SPSS Inc., Chicago). The Kolmogorov–Smirnov test indicated a parametric distribution for the biochemical parameters ($P < 0.05$). Descriptive statistics were summarised and reported as mean, standard deviation (SD), and median. For categorical variables, the chi-square (χ^2) test was used, while continuous variables were analysed with Welch's t-test. To compare biochemical parameters between subclinical hypothyroidism patients and controls Mann–Whitney U test was employed. Additionally, the Kolmogorov–Smirnov test indicated a parametric distribution for the biochemical parameters ($P < 0.05$). Lastly, Pearson's correlation was used to assess associations between hs-CRP, IL-18, and TNF-α. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of (n=450) participants were recruited for the current research, comprising 200 cases and 250 healthy, disease-free controls. The average age of the patients was reported as mean ± SD, (38 ± 3.81). The detailed demographics of the study

participants are represented in (Table-I).

TABLE-I

Representation of the demographics data of cases and controls

Parameter	Patients (n=200)	Controls (n=250)
Age (years)	38 ± 3.81	39 ± 5.4
Height (m)	1.49 ± 0.07	1.45 ± 0.07
Weight (kg)	57 ± 4.0 *	52 ± 9.1
BMI (kg/m²)	25.6 ± 4.36 *	24.7 ± 5.15
Smokers, n (%)	57 (29) **	48 (19)
Marital status (married/ unmarried)	175/25 *	185/65
Family history of autoimmune disorders, n (%)	101 (50) **	57 (23)
Parity status (nulliparous/ multiparous)	14/186	27/213

Age, height, weight, and BMI are stated as Mean ± SD, whereas smoking status, marital status, family history, and parity status are stated as n (%). BMI Body Mass Index, SD: Standard Deviation; and asterisks represent the significant difference ($p<0.01$).

According to the statistical results, BMI was significantly greater ($p<0.05$), in patients (cases) compared to the controls. Additionally, the proportion of smokers was higher among patients than controls ($p<0.01$). It was also observed that a positive family history of autoimmune diseases was more frequent in cases ($p<0.01$). Furthermore, serum levels of TSH and TPO antibody were found to be significantly elevated in cases ($p<0.001$), while significantly reduced levels of fT4, fT3 ($p<0.01$), and serum 25(OH)-Vit-D ($p<0.001$) were observed in cases in comparison to control shown in Table-II.

TSH: Thyroid Stimulating Anti-body; fT4: Free thyroxine; fT3: Free Tri-iodothyronine; TPO: Thyroid Peroxidase; 25(OH)Vit-D:25-hydroxy vitamin-D. Values of serum concentrations are represented as Mean±SD: Standard Deviation; The significance level ($p < 0.01$) was achieved using Mann–Whitney U test.

The Pearson correlation analysis revealed a significant negative correlation between the serum TSH levels and the other two biochemical factors, TPO antibodies with the serum 25 (OH) Vit-D level ($p<0.0001$). However, a slightly significant positive correlation was observed between fT4 and serum 25 (OH) Vit-D level ($p<0.001$). It was also observed that fT3 did not show any significant correlation with the levels of serum 25 (OH) Vit-D (Table-III).

TABLE-II

Representation of biochemical parameters

Parameters	Unit	Cases n=200 Mean± SD	Controls n=250 Mean± SD	P-Value
TSH	mIU/L	5.221 ± 3.12**	1.82 ± 1.51	<0.001
fT4	pmol/L	12.9 ± 5.21**	15.1±3.51	<0.01
fT3	pg/ml	1.82 ± 0.25 **	2.31±0.32	<0.01
TPOAb	IU/mL	267.7± 118**	4.7±4.91	<0.001
25(OH) Vit-D	nmol/L	26.1 ±2.5**	32.3±3.1	<0.001

TABLE-III

Pearson's Correlation between the Serum 25(OH)-Vit-D levels, thyroid function tests and TPOAb antibodies in sub-clinical hypothyroid patients (n=200)

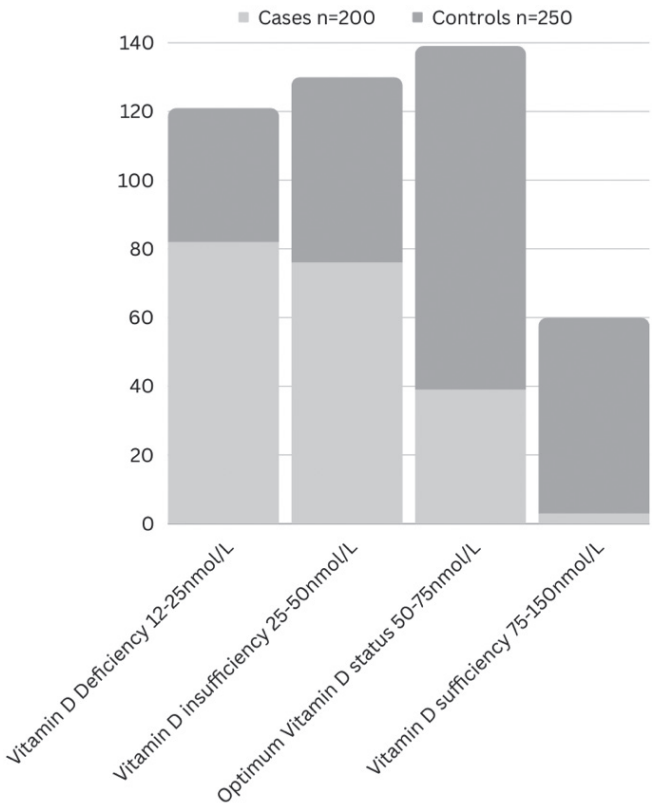
Parameters	Serum Vit-D levels (r)	P-Value
TSH	-0.658**	0.0001
fT4	0.361**	0.0001
fT3	0.121	0.11
TPO antibodies	-0.723**	0.0001

TSH: Thyroid Stimulating Anti-body; fT4:Free thyroxine;fT3: Free Tri-iodothyronine; TPO: Thyroid Peroxidase; SD: Standard Deviation;25(OH)Vit-D:25-hydroxy vitamin-D
r, Pearson's correlation rank coefficient. **$p < 0.01$.

The comparison of different strata of Vit-D deficiency levels in cases (n=200) and controls (n=250) is shown in Figure-1.

FIGURE-1

Comparison of Vitamin-D deficiency levels in cases and controls



DISCUSSION

The findings of our study revealed that the BMI of the patients was significantly higher than that of the disease-free controls. This is an interesting observation, as weight gain in sub-clinical hypothyroidism is relatively uncommon. However, a recent study, which is in agreement with our findings, states that obesity is associated with sub-clinical hypothyroidism.[9] A probable explanation for this association could be the alteration of metabolism via the thyroid auto-antibodies, which are simultaneously affected in both obesity and sub-clinical hypothyroidism.[10] In comparison to controls, hookah smoking was found to be significantly higher in sub-clinical hypothyroidism cases. This is contrary to a previous study, which demonstrated no such association.[11] However, smoking has been shown to increase oxidative stress in the body by increasing the level of reactive oxygen species. Studies have shown a considerable effect of oxidative stress on thyroid hormone[12], which may have led to this finding in the current study.

Furthermore, a noteworthy inverse correlation between vitamin-D levels and TSH among female patients with subclinical hypothyroidism was observed. Previous research has suggested a potential role of vitamin D in modulating thyroid function, and our results support this hypothesis.[13] The inverse relationship observed between vitamin-D levels and TSH levels indicates that lower vitamin-D levels may be a potential contributing risk factor for thyroid dysfunction, possibly through a common receptor pathway.[14] Another possible explanation could be the negative feedback effect of decreased Vitamin D levels on the pituitary gland, leading to increased production of thyroid-stimulating hormone, similar to its effect of parathormone.[15]

Furthermore, our study demonstrated a significantly high level of TPO antibodies in the cases, suggesting an autoimmune component in the development or progression of sub-clinical hypothyroidism in this population.[16] Recent studies are even suggesting an autophagy pathway in hypothyroidism, emphasizing the role of the immune pathway in thyroid disorders.[17]

There is a significant inverse correlation between 25 (OH) Vitamin D levels and TPO antibodies in patients with subclinical hypothyroidism in our study. A similar correlation has been previously observed in patients with Graves' disease.[18] Contrary to this, the analysis revealed fT4 and vitamin D being in a significant positive correlation in the patients of sub-clinical hypothyroidism. Few of the reported data have primarily demonstrated the effect of the vitamin D levels on the serum TSH levels independent of the thyroid hormone (T3 and T4) levels.[19] However, some experimental studies in diabetic rats have demonstrated that administration of vitamin D3 increases Dio2 expression in the liver and brain, resulting in elevated fT3 levels and reduced fT4 levels. The geographical context of Pakistan adds an interesting dimension to our study. The region's unique environmental factors, dietary habits, and sunlight exposure may contribute to variations in vitamin D levels among the population. Understanding these regional nuances is crucial for tailoring interventions and treatment strategies for sub-clinical hypothyroidism.

The implications of our findings extend beyond mere associations, as they offer insights that could inform clinical practice. Considering the high prevalence of subclinical hypothyroidism in females, especially in regions with limited sunlight exposure, routine assessment of vitamin D levels may be crucial. Additionally, monitoring TPO antibodies could help identify individuals at risk of autoimmune thyroid dysfunction.

LIMITATIONS

Nevertheless, our study has its limitations, including the small sample size. The cross-sectional design also limits our ability to establish causation, and further longitudinal studies are warranted to validate our findings. Additionally, the generalizability of our results may be limited to the specific population studied. Therefore, future studies with a larger cohort and representation from the other provinces could potentially strengthen the validity of this preliminary study.

CONCLUSION

In conclusion, our investigation into the association between vitamin D, thyroid function tests, and TPO antibodies in female patients with sub-clinical hypothyroidism in Northern Pakistan contributes valuable insights to the existing body of knowledge. The identified correlations emphasize the importance of comprehensive thyroid assessments, including vitamin D levels, in the clinical management of subclinical hypothyroidism, particularly in regions with unique environmental characteristics. Thus, as per future implications, further studies on the mechanistic underpinnings of the studied associations with potential interventions could benefit subclinical hypothyroidism patients. It will also help to investigate the Vitamin-D supplementation role as a potential adjunct therapy for sub-clinical hypothyroidism for lowering of serum TSH and TPO antibodies, leading to alleviation of dormant symptoms. This will also save the patient from Levothyroxine over-treatment and potential toxicity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Wafa Omer:** Data collection.
2	**Omer Jamshed Khan:** Concept of study.
3	**Riaz Shahbaz Janjua:** Manuscript writing.
4	**Muhammad Umar:** Proof reading.
5	**Saira Karimi:** Data analysis.