RESEARCH PAPER

"The Role of Vitamin D And VDR Gene Polymorphism (Fok1) of Genetic Susceptibility for Thyroid Cancer in North Indian Population"

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Abstract:

Thyroid cancer is the most common endocrine malignancy with a rapidly rising global incidence and a strong genetic predisposition, particularly among first-degree relatives. Most cases originate from thyroid follicular cells, mainly as papillary and follicular carcinomas, involving key pathways such as MAPK and PI3K/AKT. Genetic polymorphisms in thyroid-related genes significantly influence individual susceptibility, emphasizing the role of molecular genetics in its development. Vitamin D has anti-proliferative, pro-differentiation, and immunomodulatory effects, and its deficiency is associated with increased cancer risk. Variations in the vitamin D receptor (VDR) gene, especially Fok1 polymorphism, may modify vitamin D signaling and contribute to thyroid cancer susceptibility, particularly in North Indian populations.

Aim and Objective

The primary aim of this study was "Evaluation of Vitamin D , VDR Gene Polymorphism (FokI) as Genetic Risk Factors for Thyroid Cancer in the North Indian Population.

The study focused on these objectives:

To analyze the genetic polymorphism of the Vitamin D3 (Fok1) gene for thyroid cancer in the North Indian population.

Methodology

This case control study was conducted on 84 patients (42Cases & 42 Control**)** attending the OPD of the Department of General Surgery and was performed in the Department of Pathology & Department of Biochemistry of Santosh Medical College & hospital, Ghaziabad, Uttar Pradesh, India. Chi-square tests, t-tests, and p-values were used in the statistical analysis to determine significance.

Results

Cases were younger than controls ($p = 0.047$), with higher proportion of female. VDR (Fok1) genotype distribution differed significantly ($p < 0.0001$), with the ff genotype increasing disease risk and Ff showing a protective effect. Cases also had lower FT3 and FT4 and higher TSH levels ($p < 0.05$), indicating that VDR polymorphism and thyroid dysfunction may contribute to disease susceptibility.

Conclusion

VDR (Fok1) polymorphism is significantly associated with thyroid cancer risk, with the ff genotype increasing susceptibility and Ff showing a protective effect. Altered thyroid function (low FT3/FT4 and high TSH) further contributes to disease progression. Overall, the findings highlight a gene–environment interaction and suggest VDR polymorphism and TSH as potential markers for risk stratification.

Keywords: Thyroid cancer, VDR polymorphism Fok1, Thyroid stimulating hormones, Vitamin D.

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Introduction

Thyroid cancer is the most common endocrine malignancy with a rapidly increasing global incidence, and shows strong genetic predisposition, especially among first-degree relatives.Most thyroid cancers arise from thyroid follicular cells, predominantly as papillary and follicular thyroid carcinomas, involving key oncogenic pathways such as MAPK and PI3K/AKT.Genetic polymorphisms in thyroid-related and regulatory genes play a crucial role in individual

susceptibility to thyroid cancer, highlighting the importance of molecular genetics in its etiology. Vitamin D exhibits anti-proliferative, pro-differentiation, and immunomodulatory effects, and its deficiency has been linked to an increased risk of various malignancies, including thyroid cancer.Vitamin D receptor (VDR) gene polymorphisms, particularly FokI, may alter VDR activity and vitamin D signaling, potentially influencing genetic susceptibility to thyroid cancer in specific populations such as North Indians.

-Importance: We observed that Vitamin D status and VDR gene polymorphism (FokI) are associated with genetic susceptibility to thyroid cancer and may aid in improved patient management and reduced mortality.

Material & Methods

Study design: The study was carried out on patients of 18-65 years who were undergoing attending the OPD of the Department of General Surgery and was performed in the Department of Pathology & Department of Biochemistry of Santosh Medical College & hospital, Ghaziabad, Uttar Pradesh, India.

Place of the study: This study was carried out in the Department of Biochemistry of Santosh Medical College & hospital, Ghaziabad, Uttar Pradesh, India.

Study design: Case- Control Study.

Sample Size: Total cases will be 84 patients (42Cases & 42 Control).

- Based on previous study by Cocolos et al. (2022)[1], by taking P1= 46%, P2= 15.92% with 95% of confidence and we got a sample size of 42 patients in each groups (cases & control) of thyroid cancer.

Formula used for sample size calculation:

N (each group) $= (z_1 - \alpha/2 \sqrt{(p_1 + p_2)}\ (q_1 + q_2)/2 + z_1 - \beta \sqrt{p_1 q_1 + p_2 q_2}\ )\ (p_1 - p_2)^2$

- Hence, total cases will be 84 patients (42Cases & 42 Control).

Inclusion crieteria:

- Every adult between the ages of 18 and 65, regardless of gender, will be part of the study. Those who are diagnosed with thyroid cancer will be identified based on their pathology reports and histopathology examinations .
- Equal number of healthy individual having similar age and sex matched will be recruited as controls

Exclusion crieteria: Autoimmune Disease, Graves Disease, Hashimoto Thyroiditis, All cancer excluded except primary thyroid cancer, Inborn error of metabolism, Galactosemia, Phenylketonuria, Metabolic disorders , Diabetes Mellitus, Hemochromatosis

- Written consent was taken for all the study participants.
- Confidentiality for all the participants was maintained through out the study.

Methodology:

- **Investigations:**

Laboratory test parameter	**Methodology**
T3	Electro Chemiluminescent Immune Assay (CLIA) Method
T4	Electro Chemiluminescent Immune Assay (CLIA) Method
TSH	Electro Chemiluminescent Immune Assay (CLIA) Method
FokI gene polymorphism rs731236 analysis	Extraction of DNA, DNA QUANTIFICATION -:nanodrop spectrophotometer (Thermo Fisher Scientific Waltham, MA), -FokI VDR polymorphism, Process of DNA Agarose Gel Electrophoresis

Statistical Analysis:

"Data were analyzed using IBM SPSS Statistics (version 22.0). Descriptive statistics, including

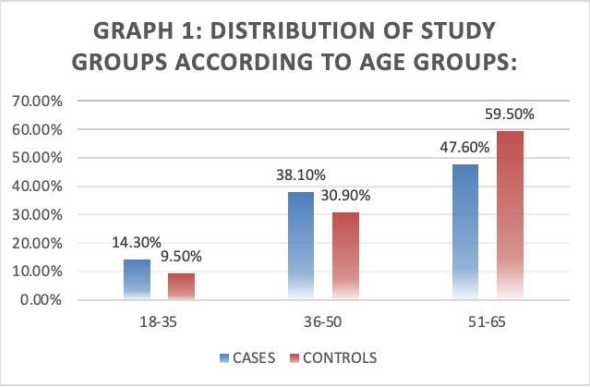
frequency and percentage for categorical variables and mean ± SD for continuous variables, were applied. The unpaired t-test was used to compare two independent groups, while the Chi-square test assessed associations between categorical variables. Correlation analysis was performed where appropriate. A p-value <0.05 was considered statistically significant."

RESULTS AND OBSERVATIONS

This case–control study evaluated the role of Vitamin D- VDR gene polymorphism (Fok1) in thyroid cancer susceptibility among the North Indian population. It included 42 thyroid cancer patients and 42 age- and sex-matched controls, conducted at Santosh Medical College and Hospital, Ghaziabad.

Table 1: Distribution of study subjects according to age

AGE GROUPS	CASES (Tot=42)	CONTROLS (Tot=42)	t-value	p-value
18-35	6 (14.3%)	4 (9.5%)		
36-50	16 (38.1%)	13 (30.9%)		
51-65	20 (47.6%)	25 (59.5%)		
MEAN + SD	51.1 ± 14.44	56.89 ± 11.78	2.01	0.047

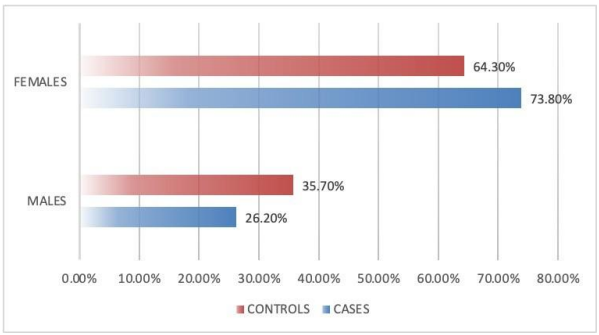


Graph 1: Distribution of study subjects according to age

Table 1, The majority of both cases (47.6%) and controls (59.5%) were in the 51–65 years age group, followed by the 36–50 years group, while the 18–35 years group constituted the smallest proportion of participants. The mean age of cases was 51.1 ± 14.44 years, whereas the mean age of controls was 56.89 ± 11.78 years. Statistical analysis showed a significant difference in age distribution between cases and controls (p = 0.047), suggesting that increasing age may be associated with the occurrence of the disease.

Table 2: Distribution of study subjects according to gender

GENDER	CASES (Tot=42)	CONTROLS (Tot=42)	x^2	p value
MALES	11 (26.2%)	15 (35.7%)	0.89	0.34
FEMALES	31 (73.8%)	27 (64.3%)		



Graph 2: Distribution of study subjects according to gender

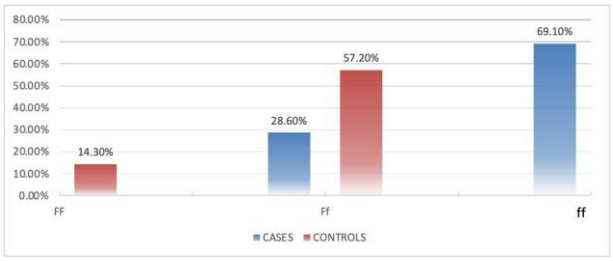
Females constituted the majority in both groups, accounting for 73.8% of cases and 64.3% of controls, while males comprised 26.2% and 35.7%, respectively. The proportion of females was higher than males in both study groups. However, the difference in gender distribution between cases and controls was not statistically significant (p = 0.34), indicating no

significant association between gender and disease occurrence in the present study.

Table 3: DISTRIBUTION OF STUDY GROUPS ACCORDING TO VDR ANALYSIS (Fok1)

VDR ANALYSIS (Fokl)	CASES	CONTROLS	P-VALUE
FF	1 (23.8%)	6 (14.3%)	0.11
Ff	12 (28.6%)	24 (57.2%)	0.002
Ff	29 (69.1%)	12 (28.6%)	0.00004
TOTAL	42	42	

Graph 3: DISTRIBUTION OF STUDY GROUPS ACCORDING TO VDR ANALYSIS (Fok1)

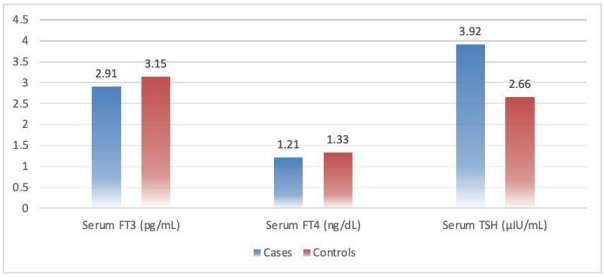


The FF genotype was observed in 23.8% of cases and 14.3% of controls, with no statistically significant difference (p = 0.11).The Ff genotype was significantly more common in controls (57.2%) than in cases (28.6%) (p = 0.002), suggesting a possible protective effect.The ff genotype was markedly higher in cases (69.1%) compared to controls (28.6%), showing a strong association with the disease (p = 0.00004).Overall, the distribution of VDR (Fok1) genotypes differed significantly between cases and controls (p < 0.0001), indicating that the ff genotype may increase disease risk.

Table 4: Biochemical Parameters for case and control subjects

Group	Serum FT3 (pg/mL)	Serum FT4 (ng/dL)	Serum TSH (µIU/mL)
Cases	2.91 ± 0.45	1.21 ± 0.25	3.92 ± 2.01
Controls	3.15 ± 0.51	1.33 ± 0.31	2.66 ± 1.18
p-value	0.00063	0.0428	0.000076

Graph 4: Biochemical Parameters for case and control subjects



Serum FT3 levels were significantly lower in cases (2.91 ± 0.45 pg/mL) compared to controls (3.15 ± 0.51 pg/mL) (p = 0.00063).Serum FT4 levels were also slightly lower in cases (1.21 ± 0.25 ng/dL) than in controls (1.33 ± 0.31 ng/dL), showing a statistically significant difference (p = 0.0428).Serum TSH levels were significantly higher in cases (3.92 ± 2.01 µIU/mL) compared to controls (2.66 ± 1.18 µIU/mL) (p = 0.000076).Overall, these findings indicate significant differences in thyroid function, with cases showing lower FT3, FT4, and higher TSH levels than controls.

Table 5: Gene polymorphism and size of PCR product

Gene	Site of Polymorphism	Type of Polymorphism	PCR Product Size	Enzyme Digestion Products	Restriction Enzyme, Site

VDR (Fok1)	**Exon 2 (Start codon)**	**T/C (F → T; f → C)**	**265 bp**	**F allele: 196 + 69 bp f allele: 265 bp (uncut)**	**Type IIS 5'-GGATG-3'**

The VDR (Fok1) gene shows a T/C polymorphism at Exon 2 (start codon), where F → T and f → C, with a PCR product size of 265 bp.After enzyme digestion, the F allele is cleaved into two fragments (196 bp and 69 bp).The f allele remains uncut, producing a single fragment of 265 bp.The restriction enzyme FokI (recognition sequence: 5′-GGATG-3′) is a Type IIS enzyme that cuts DNA at a specific distance from its recognition site.

Table 6: Genes and respective Primer sequences

Gene	Forward Primer Sequence (5′→3′)	Reverse Primer Sequence (5′→3′)
Fok1	**AGCTGGCCCTGGCACTGACTCTGGCTCT**	**ATGGAAACACCTTGCTTCTTCTCCCTC**

Table 6 lists the primer sequences used for PCR amplification of the VDR (Fok1) gene. The forward primer sequence is 5′ AGCTGGCCCTGGCACTGACTCTGGCTCT-3′ and the reverse primer is 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′. These primers specifically bind to the Fok1 gene region to enable efficient PCR amplification.

Discussion:

This case–control study suggests that Vitamin D–VDR (Fok1) gene polymorphism may contribute to thyroid cancer susceptibility in the North Indian population, supporting a role of Vitamin D–VDR pathway dysregulation in disease pathogenesis.

In Table 1, shows that most cases and controls were in the 51–65 years age group with a significant difference ($p = 0.047$), and similar mean ages, indicating adequate age matching and minimizing confounding, thereby supporting the reliability of the observed association between VDR (Fok1) polymorphism and thyroid cancer.

Consistent with findings by Pellegriti et al.(2013) and Kitahara and Sosa et al.(2016), which report a higher incidence of thyroid cancer in the fifth–sixth decades and after 40 years of age, the present study similarly observed a greater proportion of cases in the 51–65 years age group, supporting its external validity[1-2].

In the Table 2, demonstrated a clear female predominance among thyroid cancer cases, with females constituting 73.8% of affected individuals, a finding that is consistent with earlier epidemiological studies. Previous reports by Kilfoy et al. (2009) and Rahbari et al. (2010) have similarly shown a higher incidence of thyroid cancer in females, which has been attributed to hormonal influences, particularly estrogen, along with gender-specific genetic and autoimmune factors [3-4].

In our study Table 3, the ff genotype was significantly more prevalent among cases (69.1%) compared to controls (28.6%), whereas the Ff genotype was more common in controls (57.2%) than in cases (28.6%). The FF genotype showed a lower frequency in both groups, with 23.8% in cases and 14.3% in controls. The observed association ($P < 0.001$) indicates that individuals carrying the ff genotype may have an increased risk, while the heterozygous Ff genotype may exert a protective effect. This supports the hypothesis that altered VDR function may influence disease development through impaired vitamin D signaling.

In our study (Table 3), the ff genotype was significantly more prevalent among cases (69.1%) compared to controls (28.6%), while the Ff genotype was more common in controls (57.2%) than in cases (28.6%), and the FF genotype showed lower frequencies in both groups (23.8% vs 14.3%). The strong association ($P <$

0.001) suggests that the ff genotype may increase susceptibility to thyroid cancer, whereas the heterozygous Ff genotype may exert a protective effect, supporting the role of altered VDR function in disease development.

The biological basis of this association lies in the functional impact of the Fok1 polymorphism, where the F allele produces a shorter and more transcriptionally active VDR protein (424 amino acids), while the f allele results in a longer, less active receptor (427 amino acids). Consequently, individuals with the ff genotype may have reduced VDR activity, leading to impaired vitamin D signaling and diminished anti-proliferative and pro-differentiation effects, thereby contributing to carcinogenesis.

These findings are consistent with earlier reports by Uitterlinden et al.(2004) and Raimondi et al.(2009), as well as studies in Asian populations, which demonstrate a higher frequency of the ff genotype among cancer patients and highlight the influence of ethnic, environmental, and nutritional factors on VDR polymorphism, reinforcing its role in thyroid cancer susceptibility [5-6].

Thyroid function parameters are essential in evaluating thyroid disorders, and in Table 4, our study demonstrated significant biochemical alterations, with cases showing lower serum FT3 and FT4 levels and higher TSH levels compared to controls, indicating a shift toward hypothyroid or subclinical hypothyroid status and suggesting a role of altered hormone homeostasis in disease pathology; notably, the reduced FT3 levels may reflect impaired peripheral conversion or altered deiodinase activity, consistent with findings reported by Kim et al.(2015) and Boelaert.et al. (2009), supporting the idea that decreased biologically active FT3 may contribute to altered cellular differentiation and proliferation in thyroid malignancy [7,8].

Serum FT4 levels were also lower in cases compared to controls, consistent with findings by Haymart et al.(2008) and Fiore et al.(2010), suggesting compromised hormone synthesis possibly due to glandular disruption or inflammatory changes, while a key observation of this study was significantly elevated TSH levels in cases, which, as supported by Fiore and Vitti and McLeod et al., may promote thyroid carcinogenesis by stimulating cell proliferation, inhibiting apoptosis, and enhancing tumor growth [9-11].

Moreover, The observed biochemical profile, with lower FT3 and FT4 and elevated TSH levels in cases, indicates altered thyroid function associated with thyroid pathology.These findings emphasize the importance of comprehensive thyroid function assessment in thyroid disease patients. They also suggest that TSH and thyroid hormone imbalance may serve as useful biomarkers for risk stratification and monitoring. Further longitudinal and mechanistic studies are needed to establish a causal relationship with disease outcomes.

Our study evaluated the Vitamin D Receptor (VDR) Fok1 gene polymorphism, a well-characterized functional variant located at the start codon in exon 2 of the VDR gene. As shown in Table 5, the polymorphism involves a T/C nucleotide substitution, resulting in two allelic variants designated as F and f. The PCR amplification yielded a product of 265 bp, and restriction fragment length polymorphism (RFLP) analysis using the Fok1 enzyme demonstrated distinct digestion patterns for the two alleles. This methodological approach confirms the reliability of PCR-RFLP in genotyping VDR polymorphisms and aligns with established molecular diagnostic protocols used in genetic association studies.

The F allele of the VDR Fok1 polymorphism produces two fragments (196 bp and 69 bp) upon digestion, while the f allele remains uncut at 265 bp, consistent with the classical description by Gross et al.,[12], who showed that this variation results in alternative translation initiation sites yielding a shorter, more transcriptionally active VDR protein; moreover, studies such as Uitterlinden et al. and reports across diverse populations have demonstrated variable allele frequencies and reduced receptor efficiency with the f allele, supporting the reproducibility and functional significance of this polymorphism as a biologically relevant genetic variant [5]..

Several studies, including Raimondi et al.(2009), have reported a higher prevalence of the ff genotype among cancer patients, suggesting that reduced VDR activity may impair the antiproliferative and immunomodulatory effects of vitamin D; similarly, the uncut 265 bp fragment observed for the f allele in the present study supports its association with increased

disease risk, while the overall molecular profiling showing distinct PCR and digestion patterns aligns with previous literature and highlights the functional significance of VDR Fok1 polymorphism in influencing vitamin D signaling and disease susceptibility [6].

In Table 6, utilized specific forward and reverse primer sequences for the amplification of the FokI polymorphic region of the Vitamin D Receptor (VDR) gene, enabling accurate genotyping through PCR analysis. The primer pair employed was designed to flank the polymorphic site at the translation initiation codon of the VDR gene, a region known to influence receptor structure and function. The specificity and length of these primers are consistent with previously validated protocols, ensuring reliable amplification and minimizing non-specific binding.

The Fok1 polymorphism is biologically significant as it creates an alternative start codon, producing either a shorter, more transcriptionally active VDR protein (F allele) or a longer, less active variant (f allele), and studies by Gross et al.(1998) and Uitterlinden et al.(2004) have shown that this functional difference can affect vitamin D signaling, calcium metabolism, immune regulation, and cellular proliferation, contributing to disease susceptibility; furthermore, the primer sequences used in the present study are consistent with those reported in earlier population-based studies such as Raimondi et al.(2009), supporting methodological reliability and strengthening the external validity of the findings 12,5-6].

Research in Asian and Indian populations, including studies by Bid et al. and Mishra et al.(2005), has emphasized the importance of standardized primer selection to account for population-specific genetic diversity and avoid amplification bias,[13], and the robust performance of primers in the present study supports their suitability for ethnically diverse cohorts; overall, the use of well-established and functionally relevant primer sequences ensures reliable PCR amplification and reinforces the significance of the Fok1 polymorphism as a genetic marker in vitamin D–related disease research, with future large-scale and functional studies needed to further clarify its clinical implications.

CONCLUSION:

This study, titled "The Role of Vitamin D–VDR Gene Polymorphism (Fok1) in Genetic Susceptibility for Thyroid Cancer in the North Indian Population," provides comprehensive clinicopathological, biochemical, and molecular insights into thyroid carcinogenesis. Age- and sex-wise analyses revealed that thyroid cancer predominantly affects middle-aged and older adults, particularly in the 51–65-year age group, with appropriate age matching strengthening internal validity. A female predominance among cases was also observed, consistent with known epidemiological patterns, highlighting the influence of hormonal, genetic, and autoimmune factors.

The genetic findings demonstrated a significant difference in VDR Fok1 genotype distribution, with the ff genotype markedly higher among cases and the Ff genotype more common in controls, suggesting a risk and protective role, respectively. The f allele produces a longer, less transcriptionally active VDR protein, leading to impaired vitamin D signaling and reduced antiproliferative and immunomodulatory effects, particularly under conditions of vitamin D deficiency. Additionally, cases exhibited lower FT3 and FT4 levels with elevated TSH, indicating altered thyroid function, where increased TSH may promote tumor growth, reinforcing its role as a potential biomarker and contributor to disease progression.

Methodologically, the study utilized reliable PCR-RFLP techniques with standardized primers, ensuring reproducibility and validity of genetic data. Overall, the findings highlight a complex interplay between demographic factors, thyroid function, and VDR genetic variation in thyroid cancer susceptibility, supporting a gene–environment interaction model. Although limited by sample size and case–control design, the study provides valuable evidence for risk stratification and emphasizes the need for larger, multicentric, and longitudinal studies to further clarify causal mechanisms and clinical applications.

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