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ORIGINAL ARTICLE

Correlation Between Vitamin D Levels and Dermatophytosis: A Focus on Trichophyton Infections in Tinea Pedis

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ABSTRACT

Background*: Dermatophytosis, especially Tinea pedis caused by Trichophyton species, is a growing public health concern, particularly among immunocompromised individuals. Recent studies suggest a link between vitamin D deficiency and increased susceptibility to dermatophytic infections.* ***Objective****: This study evaluates the correlation between vitamin D3 levels and Tinea pedis infections, using integrated diagnostic methods.* ***Methodology****: Fifty specimens were collected, with 39 (78%) testing positive for Tinea pedis. Identification was done using conventional methods (KOH, SDA, DTM), followed by biochemical tests and molecular PCR analysis.* ***Trichophyton mentagrophytes*** *(41.0%),* ***T. rubrum*** *(28.2%),* ***T. interdigitalis*** *(20.2%), and* ***T. verricosum*** *(10.3%) were identified. Vitamin D3 levels were categorized, and statistical analysis was conducted to explore correlations with infection severity.* ***Results****: A significant portion of infected patients showed vitamin D insufficiency ($P < 0.001$), with 40% of patients aged 51-60 showing severe deficiency (<10 ng/ml). Males exhibited slightly higher deficiency rates compared to females. In contrast, 81.8% of the control group had normal vitamin D levels, suggesting a strong association between vitamin D deficiency and Trichophyton infections.* ***Conclusions****: Vitamin D deficiency is significantly associated with a higher incidence of Trichophyton infections. Integrated diagnostic techniques improved pathogen detection. These findings support the role of vitamin D in immune defense, suggesting that vitamin D supplementation may help manage recurrent infections, particularly in high-risk individuals. Further research is needed to validate these findings and guide supplementation strategies in dermatophyte treatment.*

INTRODUCTION

Vitamin D is a crucial fat-soluble vitamin involved in various processes in the human body, and its deficiency and adequacy are common issues worldwide [1]. The human obtains Vitamin D3 through two sources: exogenous, from dietary intake or endogenous, from skin production by converting certain cholecalciferol molecules [2]. The skin of humans synthesizes vitamin D and serves as the target organ for its physiologically active form. Vitamin D influences various functions through the skin, including the proliferation, differentiation, and apoptosis of keratinocytes, as well as barrier maintenance and immunoregulatory mechanisms [3].

Several environmental factors contribute to vitamin D deficiency, including winter, lack of sun exposure, and being positioned at high latitudes [4]. The immune system typically protects the body against infections with precision. An inflammatory response is initiated through the coordinated actions of immune cells, cytokines, and signaling molecules. If the immune system fails, it can lead to serious illnesses. Common symptoms of such failures include allergic reactions, inflammatory disorders, and autoimmune diseases. Additionally, vitamin D plays an essential role in skin health. A deficiency in vitamin D increases the risk of various skin diseases [5].

Dermatophytes are the primary fungal pathogens that cause various skin diseases, which can occur anywhere on the body. These fungal infections spread through direct contact with infected individuals, animals, or soil and through indirect contact with contaminated environments. This results in a significant burden on healthcare systems [6]. Dermatophytes have been divided according to the causative agents into three distinct genera (*Trichophyton* (T), *Microsporum* (M), and *Epidermophyton* (E)) and have a predilection for keratinized tissues, where they may develop, such as hair, skin, and nails [7]. *Trichophyton spp*. represents the most common factor responsible for dermatophytosis globally [8].

The most widespread species include ***Trichophyton mentagrophytes***, ***Trichophyton interdigitale***, ***Trichophyton rubrum***, and ***Trichophyton tonsurans*** [9]. These causative agents can penetrate keratinized tissues, including the outermost layer of the epidermis known as the stratum corneum [10] and the infections can range in clinical severity from mild to severe, depending on the host's immune system, the virulence of the strain, and various environmental factors. Dermatophytosis affects people worldwide, but these are more common in tropical regions due to higher temperatures and humidity [11].

Dermatophytosis requires the production of lytic enzymes and the intake of food for fungi to utilize human and animal tissues. dermatophytes must meet four criteria to invade a healthy human host: they need to grow at the body temperatures of humans or animals, bypass surface barriers, cause tissue damage and absorption, and resist immune defenses, including elevated body temperatures. The mammalian immune system has evolved to respond to potential fungal pathogens [12].

The effectiveness of certain enzymes influences the preferences of different fungal races for types of keratinous tissues. For instance, the genus Epidermophyton tends to favour the tissues found in nails and skin. In contrast, the genus Microsporum prefers the skin and hair tissues. Lastly, the genus Trichophyton attacks all keratinized tissues, including skin, hair, and nails [13]. Recent research highlights the significance of molecular identification methods in accurately detecting and treating fungal infections. Polymerase Chain Reaction (PCR) and genetic analysis are vital methods for distinguishing the types of dermatophytes that cause infections [14].

METHODOLOGY

Sample Collection:

Samples were aseptically collected from 50 tinea pedis patients (both male and female) between 12th October 2023 and 28th February 2024. The samples focused on the affected foot areas, particularly between the fourth and fifth toes, with additional blood samples collected to measure serum vitamin D levels. All samples were transported to the Central Public Health Laboratory for analysis. Vitamin D levels were quantified using ELISA, with classification as follows: >30 ng/ml considered normal, 20-29 ng/ml as inadequate, 10-19 ng/ml as moderately deficient, and <10 ng/ml as severely deficient [15].

Conventional (Microscopic and Cultural) Identification:

Samples were examined microscopically with 20% KOH and cultured on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM). Incubation occurred at 22-25°C (SDA) and 30°C (DTM) for four weeks. Dermatophytes were identified by a colour change in DTM (yellow to red) and confirmed microscopically using lactophenol cotton blue staining [16].

Biochemical (Enzymatic) Identification:

This study examined the enzymatic activities of dermatophytes to aid in species identification and understand their role in pathogenesis. The keratinase activity was assessed using a keratin azure medium, where clear zones indicated keratin degradation [17, 18]. Urease activity was tested with Christensen's Urea Agar, with a positive result, as shown by a colour change from orange to pink. ***T. mentagrophytes*** was urease-positive, while ***T. rubrum*** was negative [19]. Protease activity was tested using bovine serum albumin, where clear halos indicated enzyme presence, with ***Candida albicans*** as the control [20]. Carbohydrate assimilation was assessed with the HiCarbo™ Kit, showing the isolates' ability to utilize different carbohydrates [21]. Lipid assimilation was evaluated on Tributyrin Agar, where transparent zones around colonies indicated lipolytic activity. These enzymatic profiles help identify dermatophyte species responsible for infections like Tinea pedis [22].

Molecular Identification:

Molecular identification of dermatophytes was conducted using PCR targeting the ITS regions of the rRNA gene for accurate species identification [23, 24]. Fungal DNA was extracted from mycelial blocks cultured on SDA and incubated in malt extract broth. DNA concentration and purity were assessed using a Nanodrop spectrophotometer, and electrophoretic analysis was performed using agarose gel and a Bio-Rad Gel Doc system [25]. Conventional PCR was carried out with species-specific primers targeting ITS, 28S rDNA, topoisomerase II, and chitin synthase I genes [26]. The PCR reaction included DNA, 2X red master mix, distilled water, and primers ITS-1 and ITS-4, with amplification steps: initial denaturation at 95°C for 5 minutes, 35 cycles at 94°C for 30 sec, 52°C for 45 sec, 72°C for 45 sec, and a final extension at 72°C for 5 minutes. PCR products were analyzed on a 2% agarose gel, and species identification was confirmed by comparing ITS sequences to BLAST and MycoBank databases, with ≥99% similarity confirming the species[26].

Statistical Analysis:

Data were entered, cleaned, and analyzed using SPSS version 20. The statistical significance of detection rates was assessed using frequency, percentage, and Chi-squared tests.

RESULTS

Distribution OF Vitamin D Measurements:

Table 1 and **Figure 1** showed significant differences in serum vitamin D3 levels between the Tinea pedis

patients and the control group. Among the 39 patients, only 12.8% had normal vitamin D3 levels (>30 ng/ml), while 33.3% were insufficient (20-29 ng/ml), 35.9% were moderately deficient (10-19 ng/ml), and 17.9% had severe deficiency (<10 ng/ml). In contrast, the control group (11 participants) had 81.8% with normal levels and 18.2% with insufficient levels. A significant association was found between low vitamin D3 levels and Tinea pedis infections ($p < 0.001$).

Table 1: The Measured Serum Vitamin D3 Levels among the infected Patients with Tinea pedis and control groups.

Serum Vitamin D3 Level (ng/ml)	Patients infected by Tinea pedis (%)	Control Group (%)	P-value
Normal (>30)	5 (12.8%)	9 (81.8%)	➢ ****0.001**
Inadequate (20-29)	10 (25.6%)	2 (18.2%)	0.003**
Moderately Deficient (10-19)	14 (35.9%)	0 (0%)	> **0.001
Severely Deficient (<10)	10 (25.6%)	0 (0%)	➢ **0.001
Total	39 (100%)	11 (100%)	-

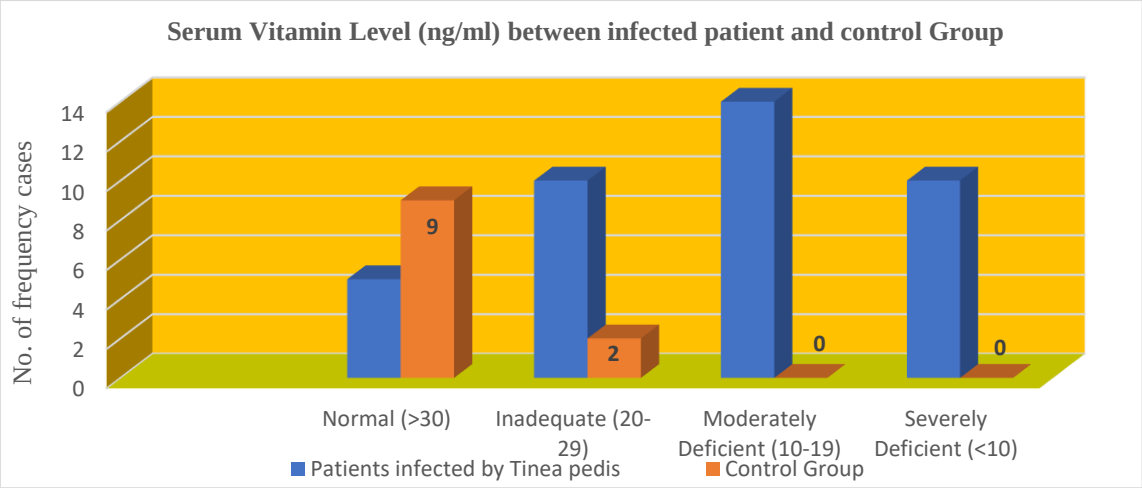

Fig. 1: The measurement of vitamin D levels and a comparison among patients infected with Tinea pedis and the negative control group.

Correlation of Vitamin D3 Levels by Age Group and Sex:

In this study of 39 patients with dermatophyte infections, severe vitamin D deficiency (<10 ng/ml) was more prevalent in men (37.5%) compared to women (20.0%), with statistical significance ($p < 0.001$). The highest deficiency rates were observed in older age groups, particularly in the 51-60 years (40%) and 41-50 years (30%) categories, highlighting a link between vitamin D deficiency and increased susceptibility to fungal infections. In contrast, the control group exhibited a higher percentage of normal vitamin D levels (81.8%), suggesting that adequate vitamin D may protect against dermatophytosis, as shown in **Table 2.**

Table 2: Correlation between the distribution of Vitamin D with age group and sex.

	Age groups						Sex		
Serum Vitamin D3 rates (ng/ml)	**>20**	**21-30**	**31-40**	**41-50**	**51-60**	**>60**	**Female (n=15)**	**Male (n=24)**	**P-value**
Normal (>30)	1	1	1	1	1	0	2(13.3%)	3(12.5%)	<0.05
Inadequate (20-29)	1	2	2	2	2	1	5(33.3%)	5(20.8%)	<0.05
Moderately Deficient (10-19)	1	2	3	3	3	2	5(33.3%)	7(29.2%)	<0.01
Severely Deficient (<10)	0	0	2	3	4	1	3(20.0%)	9(37.5%)	<0.001

Conventional Identification:

Conventional identification methods were assessed for dermatophyte infections, as shown in **Figures 2 and 3.** The 20% KOH assay demonstrated perfect sensitivity, identifying all 50 samples (100%). Sabouraud Dextrose Agar (SDA) culture identified 92% of samples (46/50), while Dermatophyte Test Medium (DTM) had the lowest detection rate at 78% (39/50). A statistically significant difference was observed between KOH and DTM results (P = 0.002). **Fig. 3** illustrates fungal growth on media and the conventional diagnostic process for ***T. rubrum*** infections in Tinea pedis.

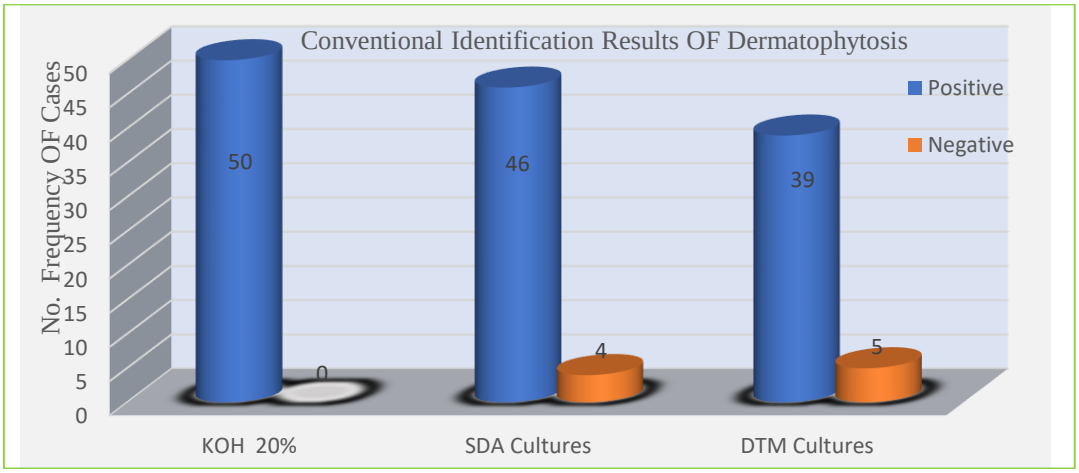

Fig. 2: The results of the conventional diagnosis for dermatophyte infections.

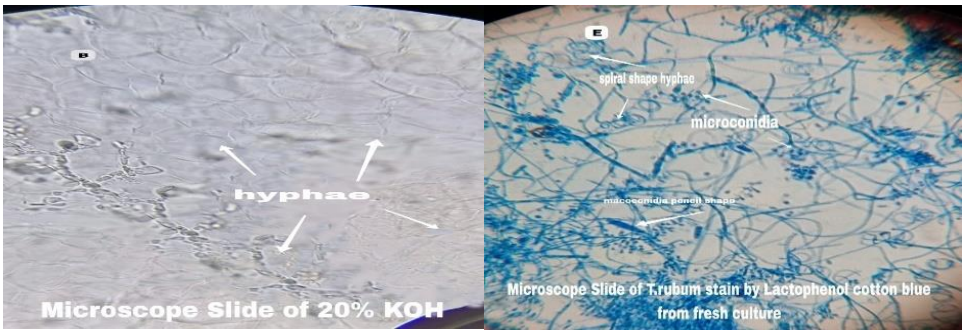

Fig. 3: The results of the series of steps to follow the conventional diagnosis procedure for ***Trichophyton rubrum*** dermatophyte infections in Tinea pedis.

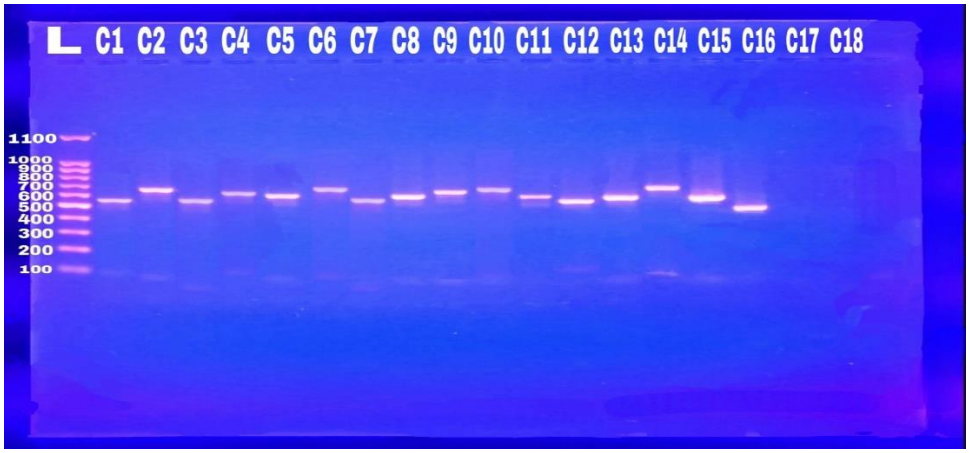

Fig. 4: Gel electrophoresis result of determining the PCR-Amplified ITS Region Size Utilize ITS1 with ITS4 Primers in 1.5% Agarose gel electrophoresis at 70 volt/ cm2 for 1:30 hrs. with UV visualization.

Biochemical Identification of Tinea Pedis:

Table 3 summarizes the biochemical profiles of 39 dermatophyte isolates. Keratinase activity was universal (100%), underscoring its role in pathogenesis. Protease activity was high (92.3%), especially in ***T. verricosum*** (100%) and ***T. mentagrophytes*** (93.8%). Urease hydrolysis was exclusive to ***T. mentagrophytes*** (100%), while ***T. rubrum*** showed none. DNase activity appeared in 71.8% of isolates, notably, ***T. mentagrophytes*** and ***T. verricosum*** (75%). Lipid and glucose utilization were lowest at 48.7% and 41.0%, respectively.

Statistical analysis showed significant differences in keratinase ($P = 0.000$), protease ($P = 0.001$), DNase ($P = 0.027$), and urease ($P = 0.038$), while carbohydrate and lipid metabolism differences were non-significant ($P > 0.05$).

PCR Amplification Techniques Results:

In **Table 4,** PCR confirmed *Tinea pedis* identification, especially in vitamin D-deficient patients. Gel electrophoresis showed bands (550–690 bp), indicating ***T. mentagrophytes*** (41.0%), ***T. rubrum*** (28.2%), ***T. interdigitalae*** (20.5%), and ***T. verricosum*** (10.3%), with significant statistical differences for some species ($P < 0.05$).

Correlation Between Dermatophyte Species Infection and Vitamin D3 Deficiency Levels:

Figure 5 highlights a significant correlation ($P < 0.05$) between low Vitamin D3 levels and Trichophyton infections. The highest prevalence was found in patients with moderate to severe deficiency (<20 ng/mL), particularly ***T. rubrum*** (72.8%) and ***T. verricosum*** (75%). In contrast, only 10.3% of infections were observed in individuals with normal Vitamin D levels (≥30 ng/mL).

Table 3: The results of the dermatophyte tinea pedis infections by *Trichophyton spp*. identified by biochemical tests are revealed.

Biochemical identification	*T. mentagrophytes* (N=16) (%)	*T. rubrum* (N=11) (%)	*T. interdigitalae* (N=8) (%)	*T. verrucousm* (N=4) (%)	Total	*P-value*
keratinase activities assays	16 (100%)	11 (100%)	8 (100%)	4 (10%)	39(100%)	0.0001(S)
Urease hydrolysis activities assays	16 (100%)	0 (0%)	4 (50%)	2 (50%)	22(56.6%)	0.038(S)
DNase activities assays	12 (75%)	8 (72.7%)	5 (62.5%)	3 (75%)	28(71.8%)	0.027(S)
Protease activities assays	15 (93.8%)	10 (90.9%)	7 (87.5%)	4 (100%)	36(92.3%)	0.001 (s)
Carbohydrates Assimilation Tests	7 (43.8%)	5 (45.5%)	2 (25%)	2 (50%)	16(41.0%)	0.078(NS)
Lipids Assimilation Tests	8 (50%)	6 (54.4%)	3 (37.5%)	2 (50%)	19(48.7%)	0.068(NS)

Table 4. Frequency distribution of dermatophyte agents in Tinea pedis according to genetics sizes.

No.	Causative agents	ITS region (bp)	Frequency (N) (%)	P-value
1	***Trichophyton mentagrophytes***	550 bp ~	16 (41.0%)	**0.004
2	***Trichophyton rubrum***	690 bp ~	11 (28.2%)	*0.021
3	***Trichophyton interdigidalae***	617 bp ~	8 (20.5%)	*0.032
4	***Trichophyton verrucosm***	650 bp ~	4 (10.3%)	0.079
5	**Total**	-	39 (100%)	-

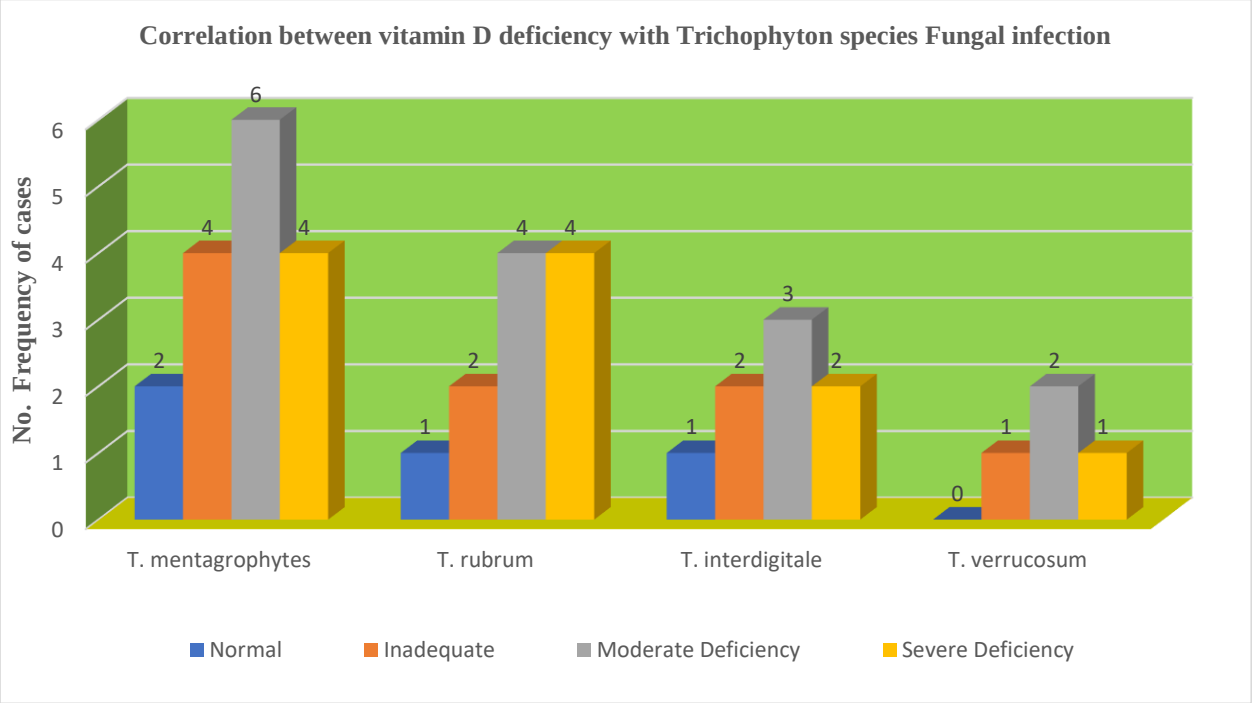

Fig. 5: The association between the distribution of the infection by dermatophytes ***Trichophyton species*** among vitamin D deficiency patients.

DISCUSSION

Overcrowding, and global travel. Among the most critical risk elements identified are human migration, animal contact, and environmental exposure. A notable underlying factor is vitamin D deficiency, which the World Health Organization reports affects over 2 billion individuals globally [27]. Given vitamin D's established role in skin integrity, immune regulation, and epithelial repair, its deficiency presents a potential predisposition to fungal skin infections [28].

This study demonstrated a strong association between vitamin D deficiency and dermatophyte infections, particularly those caused by *Trichophyton* species, such as ***T. rubrum*** and ***T. mentagrophytes*****.** Of the 50 clinical samples examined, 39 tested positive for dermatophytes. The most prevalent species included ***T. mentagrophytes***, ***T. rubrum***, ***T. interdigitalis***, and ***T. verricosum***. Notably, vitamin D deficiency was more pronounced among infected individuals, especially older adults. The immunoevasive nature of ***T. rubrum*** may partially explain its higher incidence in vitamin D-deficient patients [29, 30].

Diagnostic confirmation was achieved through a combination of traditional and molecular approaches. KOH microscopy, SDA culture, and DTM testing successfully identified dermatophyte presence, while biochemical assays (e.g., protease, keratinase, urease, DNase activity, and nutrient utilization) supported their pathogenic potential. Molecular identification via PCR further validated the predominance of ***T. mentagrophytes*** and ***T. rubrum***, underscoring the utility of PCR as a sensitive and specific diagnostic tool [31,32].

Statistical analysis revealed a significant disparity in vitamin D levels between infected patients and healthy controls ($p < 0.001$). Severe deficiency (<10 ng/ml) was most prevalent among infected individuals aged 51–60. Additionally, a slight gender difference was observed, with men showing higher rates of insufficiency than women [33,34]. These patterns are in line with previous findings supporting the role of vitamin D in modulating host immunity and potentially mitigating fungal infections. Ensuring adequate vitamin D status may contribute to both the prevention and attenuation of dermatophyte-related disease severity [35].

Regarding species distribution, statistical significance was observed ($p < 0.05$), with ***T. mentagrophytes*** emerging as the dominant species (41.0%, $p = 0.004$). Its adaptability to both human and animal environments explains its high prevalence, as also reported by Assi *et al*. [36]. ***T. rubrum*** (28.2%) was more commonly associated with chronic and relapsing infections, especially in warm and humid settings[14]. ***T. interdigitalis*** (20.5%) was frequently identified in individuals exposed to communal showers and swimming facilities, aligning with Segal *et al*.[37]. Finally, ***T. verricosum*** (10.3%) was linked to zoonotic transmission, supporting earlier findings by Moskaluk *et al*.[38].

CONCLUSION

This study links vitamin D deficiency to increased susceptibility to dermatophytosis, especially *Tinea pedis*, with *Trichophyton* species as primary pathogens. Lower vitamin D levels, particularly in older individuals, are associated with higher infection rates. Diagnostic methods, including biochemical and PCR techniques, identified ***T. mentagrophytes, T. rubrum,*** as well as other *Trichophyton* species.

Vitamin D plays a key role in immune defense, suggesting its potential as a therapeutic adjunct for fungal infections. Vitamin D supplementation could aid in managing chronic infections, though further research is needed to confirm causality, optimal dosages, and long-term benefits. Future studies should explore its role in preventing and treating dermatophytosis, especially in high-risk groups.

Declarations:
Ethics approval and consent to participate: This study was performed at the College of Health and Medical Techniques and approved by the same college Research Ethics Committee, which is a member of (Approval number: R 13/2324 Date:15-9-2023).
Availability of data and material: Available
Conflict of interests: No
Funding: No
Authors contributions:
All authors have read and approved the manuscript.
Author 1: Literature Review, Writing & Critical Review.
Author 2: Conception, Design & Supervision. Analysis and/or Interpretation.
Author 3,4: materials, Data collection and/or Processing& Literature Review.
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