

Serum Vitamin D3 and Zinc Levels in Patients with Vitiligo

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Abstract

Background: Vitiligo is a chronic skin condition that results in the loss of skin pigmentation due to the loss of functional melanocytes. Micronutrients, including zinc and vitamin D, along with inflammatory mediators, have been shown to impact immune response and skin health.

Objectives: To evaluate serum zinc and vitamin D levels in patients with vitiligo and healthy controls and to assess the relationship between these parameters and other biochemical markers.

Methods: A case-control study was conducted at the Dermatology Department, Baghdad Teaching Hospital, Baghdad, Iraq (November 2025 – April 2026), enrolling 70 patients with vitiligo and 50 healthy controls. Serum Vitamin D3 was measured using an electrochemiluminescence immunoassay (Elecsys Vitamin D Total III, Roche Diagnostics), and serum zinc was measured using a direct colorimetric method (Spinreact Zinc Color 5-Br-PAPS kit).

Results: Vitamin D3 was highly and significantly lower in patients (15.51 ± 0.739 vs. 25.90 ± 1.458 ng/mL; $p < 0.001$). Zinc showed a borderline lower level in patients (82.19 ± 2.143 vs. 96.08 ± 6.605 mcg/dL; $p = 0.058$). Vitamin D3 demonstrated excellent ROC performance (AUC=0.813; 95% CI: 0.738–0.888; sensitivity 95.7%; specificity 72.0% at ≤ 26.5 ng/mL cut-off). Zinc showed no significant ROC performance (AUC=0.504).

Conclusions: Vitamin D3 and zinc deficiencies are the most consistent and clinically significant biochemical abnormalities in vitiligo, independent of sex, suggesting an important role for vitamin D deficiency in the disease process. Vitamin D3 and zinc did not show significant correlations with the inflammatory markers, suggesting that nutritional imbalance and inflammation may run in parallel, not as the same process.

مستويات فيتامين دي 3 والزنك في مصل الدم لدى مرضى البهاق

سجى عبد الناصر، انتظار رفعت سرحت

الخلاصة

الخلفية: البهاق هو حالة جلدية مزمنة تؤدي إلى فقدان تصبغ الجلد نتيجة فقدان الخلايا الصبغية الوظيفية. وقد ثبت أن المغذيات الدقيقة، بما في ذلك الزنك وفيتامين د، إلى جانب الوسائط الالتهابية، تؤثر على الاستجابة المناعية وصحة الجلد. الأهداف: تقييم مستويات الزنك وفيتامين د في مصل الدم لدى مرضى البهاق والأصحاء، وتقييم العلاقة بين هذه المعايير والمؤشرات البيوكيميائية الأخرى.

طرق البحث: أجريت دراسة حالات وشواهد في قسم الأمراض الجلدية، مستشفى بغداد التعليمي، بغداد، العراق (تشرين الثاني 2025 – نيسان 2026)، شملت 70 مريضاً بالبهاق و50 شخصاً من الأصحاء كمجموعة ضابطة. تم قياس فيتامين د3 في المصل باستخدام مقايصة التآلق الكهروكيميائي المناعي (Elecsys Vitamin D Total III)، روش دياغنوستيكس)، وتم قياس الزنك في المصل باستخدام طريقة قياس اللون المباشرة (مجموعة Spinreact Zinc Color 5-Br-PAPS).

النتائج: كان فيتامين د3 أقل بشكل ملحوظ وذو دلالة إحصائية عالية لدى المرضى (0.739 ± 15.51 مقابل 25.90 ± 1.458 نانوغرام/مل؛ $p < 0.001$). أظهر الزنك مستوى أقل بشكل حدي لدى المرضى (2.143 ± 82.19 مقابل 6.605 ± 96.08 ميكروغرام/ديسيلتر؛ $p = 0.058$). أظهر فيتامين د3 أداءً تشخيصياً ممتازاً في منحنى ROC ($AUC = 0.813$)؛ فاصل الثقة 95%: $0.888 - 0.738$ ؛ الحساسية 95.7%؛ النوعية 72.0% عند نقطة القطع ≥ 26.5 نانوغرام/مل). لم يُظهر الزنك أداءً تشخيصياً ذا دلالة إحصائية ($AUC = 0.504$).

الاستنتاجات: يُعدّ نقص فيتامين د3 والزنك من أكثر الاضطرابات البيوكيميائية اتساقاً وأهمية سريرية في البهاق، بصرف النظر عن الجنس، مما يشير إلى دور مهم لنقص فيتامين د في العملية المرضية. لم يُظهر فيتامين د3 والزنك ارتباطات ذات دلالة إحصائية مع المؤشرات الالتهابية، مما يشير إلى أن اختلال التوازن الغذائي والالتهاب قد يسيران بشكل متوازٍ وليس كعملية واحدة.

1. Introduction

Vitiligo is a chronic skin condition that results in the loss of skin pigmentation that is a result of the loss of functional melanocytes. This presents as white patches of skin that can occur on skin and mucosal surfaces as well. Vitiligo is on the spectrum of the most common skin disorders as it affects 0.5–2% of the world's population. There is no predilection for gender or race. This skin condition can occur in people of all ages, but it is most seen in people under 30. Vitiligo can have a strong psychosocial impact on people who have this condition due to the stigma that skin discoloration carries (Jan & Masood, 2023; Felsten, Alikhan & Petronic-Rosic, 2011; Lee et al., 2019). Vitiligo affects a small but significant part of the world population. There are a number of factors that are known to influence vitiligo and include genetic susceptibility, autoimmune responses, oxidative stress, and environmental triggers (Mahmood, Sulaiman & Sakran, 2022). Autoimmunity is seen as the most prominent contributor to the destruction of melanocytes and is seen as the most significant in the case of vitiligo. Numerous studies have shown that the immune system causes the production of inflammatory mediators which cause the destruction of melanocytes and result in their death. This has led to a greater search for further identifying inflammatory mediators involved in the development and worsening of vitiligo (Mahmood, Sulaiman & Abass, 2022). Micronutrients including zinc and vitamin D along with inflammatory mediators have also been impacting immune response and skin health. Zinc is an immune modulator and is also involved in antioxidant defense and the function of melanocytes. Zinc deficiency can lead to poor immune response and can worsen a number of skin conditions, including vitiligo (Rodrigues et al., 2017; Mogaddam et al., 2017; Al-Khafaji, Brito & Bin, 2022). Vitamin D has immune modulating effects and can also affect the survival of melanocytes and the pigmentation of the skin. Autoimmune diseases are associated with low levels of vitamin D, and this deficiency can also contribute to the onset of vitiligo (Bagherani, 2015). Research on inflammatory markers and micronutrients in autoimmune disease processes continues to be of interest. The interplay between zinc and vitamin D for vitiligo has yet to be fully studied. This necessitates further study to assess the connection of the micronutrients and vitiligo. This research aimed to understand the underlying factors contributing to the biochemical changes occurring in vitiligo patients. In addition to advancing the current knowledge about vitiligo by finding new biomarkers, it may help clinicians in diagnosing vitiligo, disease management, and in defining the treatment targets.

2. Materials and Methods

2.1. Study Design and Subjects

This case-control study included 120 participants, comprising 70 patients diagnosed with vitiligo and 50 controls. Patients were recruited from the Dermatology Department, Baghdad Teaching Hospital, Baghdad, Iraq, during the study period from November 2025 to April 2026. The patient group comprised individuals who were clinically diagnosed with vitiligo by a dermatologist. The control group comprised individuals with

no personal history of vitiligo or other autoimmune or chronic inflammatory disorders. The control participants were selected in a manner that they were of comparable age and sex to the patient participants. All study participants were informed of the study objectives and the type of samples that would be collected from them. They were informed that their participation in the study was voluntary, and they were assured of their right to privacy and confidentiality regarding their participation and the study in general.

2.2. Inclusion Criteria

The inclusion criteria included patients who had been clinically diagnosed with vitiligo by a specialist dermatologist, as well as apparently healthy subjects of both sexes demonstrating a wide age range who were living in Iraq.

2.3. Exclusion Criteria

Participants with other autoimmune diseases and those with acute or chronic inflammatory diseases were also excluded from the study. Patients with any marked liver, kidney, cancer, or other metabolic diseases, pregnant women, and participants who were on immunosuppressive therapy or other therapy were excluded.

2.4 Sample Size and Power Analysis

Sample size determination was performed using G* Power version 3.1.9.7, estimating the sample size based on the t-test for the difference between two independent means. With a moderate effect size ($d = 0.5$), alpha error probability of 0.05, and 85% power, the calculated sample size considering equal allocation was 118 participants. The current study comprised 120 participants (70 patients with vitiligo and 50 healthy controls), which was more than the estimated total sample size and was thus deemed adequate for statistical analysis.

2.5 Sample Collection and Preparation

Venous blood samples were obtained from patients with vitiligo and healthy control persons. Blood was allowed to coagulate at room temperature and serum was obtained by centrifugation. The serum was kept at -20°C until the time of assay.

2.6 Analysis of Serum Vitamin D3

The Roche Diagnostics Elecsys Vitamin D Total III Assay was used to determine the serum vitamin D concentration using a Cobas e immunoassay. The Elecsys vitamin D total III assay analyzer is an example of an electrochemiluminescence immunoassay used to measure the total concentration of 25-hydroxyvitamin D in human serum samples (Khoshdel et al., 2022).

2.7 Measurement of Serum Zinc Concentration

Serum zinc concentration was measured using the Spinreact Zinc Color 5-Br-PAPS kit via a direct colorimetric method. Zinc reacts with 5-Br-PAPS in a buffered medium at pH 8.6 to produce a colored complex (Mogaddam et al., 2017). Working reagents were prepared by adding a single dose of R3 (ascorbic

acid powder) to one vial of R1 buffer and gently mixing until complete dissolution of the powder. Once prepared, the working reagent was stable for 30 days at 2–8°C when sealed tightly and protected from contamination (Bagherani, 2015).

2.8 Statistical Analysis

Statistical analyses were performed using the IBM SPSS version 27 package. Quantitative variables are expressed as mean $\pm$ SE and qualitative variables as numbers and percentages. Independent samples t-tests were used for comparisons between patients with vitiligo and healthy controls for variables with normal and non-normal distributions, respectively. For non-continuous variables, the chi-square test or Fisher's exact test was used. The relationships between variables were tested using Pearson and Spearman correlation tests. For the studied biomarkers, ROC curve analysis was performed to establish their diagnostic value(s) when applicable. Statistical significance was set at $p < 0.05$ (Kim et al., 2024; Salem et al., 2023).

3. Results

In Table1 you can see the participants' demographic information. The research included 70 vitiligo patients, 35 of whom were male and 26 of whom were female, as well as 50 healthy controls. The control group had an average age of 18.96 ± 1.574 years, while the sick group had an average age of 22.70 ± 1.298 years. The lack of a statistically significant age difference ($p=0.070$) between the two groups indicates that they were very similar to one another.

Table1: Demographic Characteristics of Vitiligo Patients and Healthy Controls

Parameter	Male Patients (n=35)	Female Patients (n=35)	Male Controls (n=24)	Female Controls (n=26)
Age (years)	22.70 ± 1.30	22.69 ± 2.01	18.96 ± 2.43	19.00 ± 1.81
n (%)	35 (50%)	35 (50%)	24 (48%)	26 (52%)
Age: patients 22.70 ± 1.298 vs. controls 18.96 ± 1.574 ; $p=0.070$ (ns). Values are Mean $\pm$ SE.				

Vitamin D3 was highly and significantly lower in patients (15.51 ± 0.739 vs. 25.90 ± 1.458 ng/mL; $p<0.001$). Zinc levels were borderline lower in patients than in controls (82.19 ± 2.143 vs. 96.08 ± 6.605 mcg/dL; $p=0.050$), as shown in Table2.

Table2: Overall Comparison of Serum Vitamin D3 And Zinc Between Vitiligo Patients and Healthy Controls

Parameter	Patients (n=70) Mean $\pm$ SE	Controls (n=50) Mean $\pm$ SE	t	P value
Zinc (mcg/dL)	82.19 ± 2.143	96.08 ± 6.605	-2.000	0.058
Vitamin D3 (ng/mL)	15.51 ± 0.739	25.90 ± 1.458	-6.353	<0.001

Sex-based subgroup analysis revealed that serum zinc levels differed significantly according to sex and study groups (86.68 ± 3.58 , 77.83 ± 2.21 , 89.46 ± 5.28 , and 102.19 ± 11.73 mcg/dL, respectively; $p=0.046$). In contrast, vitamin D3 levels were significantly lower in both male and female patients than in male and female controls (14.20 ± 1.11 , 16.83 ± 0.94 , 27.04 ± 1.96 , and 24.85 ± 2.16 ng/mL, respectively; $p<0.001$), as illustrated in Table3.

Table3: Zinc and Vitamin D3 Across Four Sex-Dependent Subgroups

Parameter	Male Patient (n=35)	Female Patient (n=35)	Male Control (n=24)	Female Control (n=26)	F	P value
Zinc (mcg/dL)	$86.68 \pm 3.58^{ab\uparrow}$	77.83 ± 2.21^a	89.46 ± 5.28^{ab}	102.19 ± 11.73^b	2.749	0.046
Vitamin D3 (ng/mL)	14.20 ± 1.11^a	16.83 ± 0.94^a	27.04 ± 1.96^b	24.85 ± 2.16^b	16.779	<0.001

Serum Zinc was the only marker to show a statistically significant difference between male and female patients (86.68 ± 3.581 vs. 77.83 ± 2.209 mcg/dL; $F=4.479$; $p=0.038$), with male patients recording higher zinc levels than female patients. Vitamin D3 levels showed a borderline trend toward higher levels in female patients (16.83 ± 0.941 vs. 14.20 ± 1.109 ng/mL; $F=3.267$; $p=0.075$) Table 4 and Fig.1 and Fig.2.

Table4: Within-Patient Sex Comparison Between Male and Female Vitiligo Patients

Parameter	Male Patients (n=35) Mean $\pm$ SE	Female Patients (n=35) Mean $\pm$ SE	F	p-value
Zinc (mcg/dL)	86.68 ± 3.581	77.83 ± 2.209	4.479	0.038
Vitamin D3 (ng/mL)	14.20 ± 1.109	16.83 ± 0.941	3.267	0.075 ns

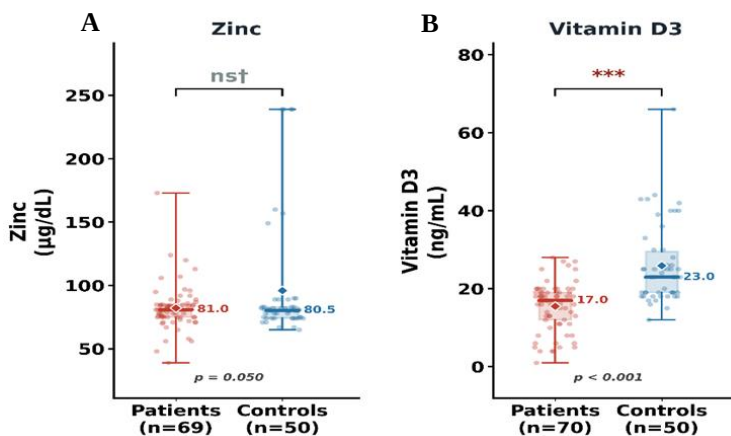


Figure1: Serum Zinc and Vitamin D3 Levels in Patients and Healthy Controls.

(A) Zinc concentrations showed no significant difference between groups ($P = 0.050$).

(B) Vitamin D3 concentrations were significantly lower in patients than in healthy controls ($***P < 0.001$). Individual data points are shown with summary statistics.

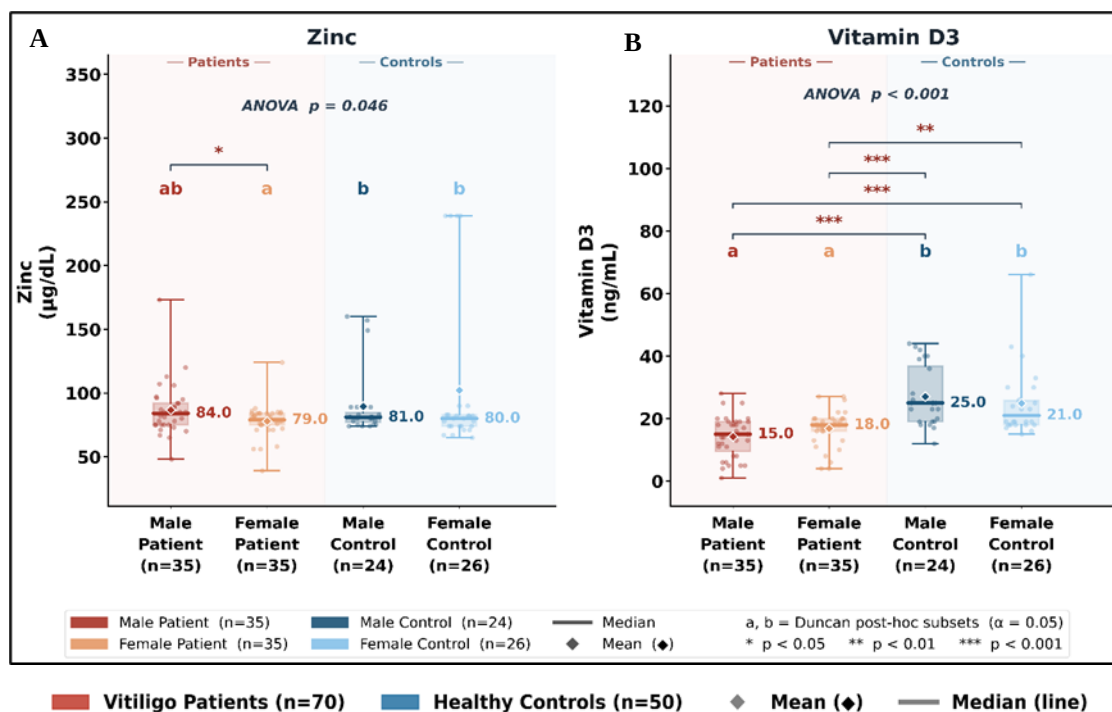


Figure2: Sex-Stratified Serum Zinc and Vitamin D3 Levels in Vitiligo Patients And Healthy Controls. (A) Serum zinc concentrations differed significantly among the study groups (ANOVA, $P = 0.046$). (B) Serum vitamin D3 concentrations were significantly lower in patients with vitiligo than in healthy controls (ANOVA, $P < 0.001$). Different letters indicate significant differences according to Duncan's post hoc test ($P < 0.05$). Individual data points are shown with the mean (♦) and median (line).

As shown in Table5, there were no statistically significant associations discovered between Zinc or Vitamin D3 and any other biomarker (all $p > 0.05$), or between Zinc and Vitamin D3 ($r = -0.209$; $p = 0.085$).

Table5: Spearman Correlation Between Zinc, Vitamin D3, and Inflammatory Markers in Vitiligo Patients

Parameter	Lipocalin-2	Amyloid A	Calprotectin	Zinc	Vitamin D3
Zinc	0.150 ns	0.066 ns	0.026 ns	—	-0.209 ns
Vitamin D3	-0.071 ns	-0.094 ns	-0.179 ns	-0.209 ns	—
Spearman's rank correlation coefficient (r).					

As shown in Table6, Vitamin D3 demonstrated excellent diagnostic performance in the inverted direction (AUC=0.813; 95% CI: 0.738–0.888; $p < 0.001$), with a cut-off of ≤ 26.5 ng/mL achieving a sensitivity of 95.7% and specificity of 72.0%, confirming that markedly lower Vitamin D3 levels are strongly characteristic

of patients with vitiligo. Zinc (AUC=0.504; 95% CI: 0.398–0.611; $p=0.938$) did not reach statistical significance, with a cutoff of ≥ 83.5 $\mu\text{g/dL}$ (sensitivity 43.5%; specificity 76.0%). As illustrated in Fig.3.

Table6: ROC Curve Analysis: Diagnostic Performance of Serum Vitamin D3 And Zinc for Vitiligo

Marker	AUC	SE	95% CI	p-value	Cut-off	Sn%	Sp%	Youden J
Vitamin D3 (ng/mL)	0.813	0.038	0.738 – 0.888	<0.001***	≤ 26.5 ng/mL	95.7%	72.0%	0.677
Zinc ($\mu\text{g/dL}$)	0.504	0.054	0.398 – 0.611	0.938 ns	≥ 83.5 $\mu\text{g/dL}$	43.5%	76.0%	0.195

Optimal cut-off at maximum Youden index ($J = \text{Sensitivity} + \text{Specificity} - 1$). *** $p < 0.001$; ns = non-significant ($p > 0.05$).

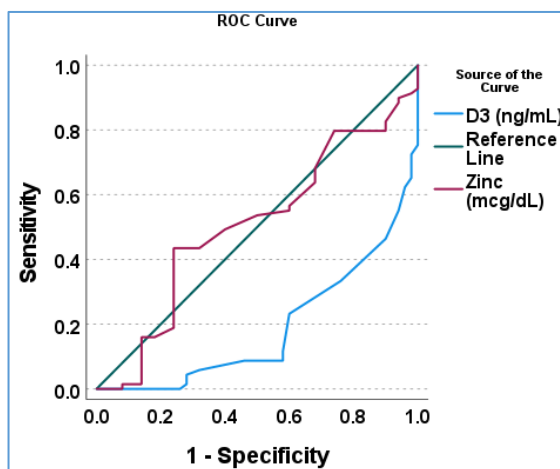


Figure3: ROC Curve Analysis of Serum Zinc and D3 For Vitiligo

4. Discussion

Vitiligo is a chronic acquired depigmentation disorder driven by selective autoimmune destruction of epidermal melanocytes, in which imbalances in innate and adaptive immune signalling, oxidative stress burden, and nutritional dysregulation converge to sustain the melanocyte-directed autoimmune process (Sri et al., 2025). The age and sex profiles of this study cohort reflect well-documented epidemiological features of vitiligo; the predominantly young patient cohort and equal sex distribution are entirely consistent with that pattern (Mahmmod & Ismael, 2021; Alikhan et al., 2011). Vitamin D3 deficiency represents the most reproducible and clinically significant single biochemical alteration associated with vitiligo in this study (Usha Sri et al., 2025). Vitamin D3 synthesized in the skin following ultraviolet B irradiation of 7-dehydrocholesterol and subsequently activated through sequential hepatic and renal hydroxylation to

calcitriol (1,25-dihydroxyvitamin D3), mediates its biological effects primarily through binding to the nuclear vitamin D receptor (VDR). The VDR is expressed on virtually all immune cell types T-lymphocytes, B-lymphocytes, dendritic cells, macrophages, and natural killer cells and its activation by calcitriol exerts broad immunomodulatory effects that are directly relevant to vitiligo pathogenesis (Mishra et al., 2019; Chang & Ko, 2023; Inoue, 2025; Marrapodi, Marini & Bellei, 2025). Calcitriol promotes immune tolerance by suppressing the differentiation and function of pro-inflammatory Th1 and Th17 subsets while simultaneously enhancing regulatory T-cell (Treg) induction and Th2 polarization, leading to reduced production of IFN- γ , IL-17, and TNF- α . Since dysregulated Th1 and cytotoxic CD8⁺ T-cell responses are the primary effectors of melanocyte destruction in vitiligo, the deficiency of Vitamin D3 and the consequent impairment of VDR-mediated immune tolerance represents a critical upstream defect that removes a fundamental brake on the autoimmune process which is reported in many studies and confirm this study findings (Manoharan, Pandian & Venkatesan, 2025; Yang et al., 2021). Calcitriol exerts direct cytoprotective effects on melanocytes by upregulating the expression of catalase, glutathione peroxidase, and other antioxidant defence proteins, and promotes melanocyte differentiation, proliferation, and survival through activation of downstream signalling pathways involving stem cell factor (SCF) and its cognate receptor tyrosine kinase c-Kit (Albazali et al., 2024; Bechrouri et al., 2019a; Bechrouri et al., 2019b). A study also confirms this study findings and reported that Vitamin D3 deficiency could serve as depigmented patches expand and functional melanocyte-containing skin area diminishes the capacity for UV-mediated cutaneous Vitamin D3 synthesis progressively declines, further deepening the deficiency and thereby compounding the immunoregulatory failure (Karagüzel et al., 2016). A study confirmed the high specificity of Vitamin D3 as a discriminator in vitiligo and demonstrated that levels above a comparable threshold are strongly associated with normal immune tolerance and melanocyte function (Sanad et al., 2020). The borderline reduction in serum Zinc in vitiligo patients relative to controls indicates that Zinc does not function as a reliable individual-level discriminator of vitiligo in this study. However, the significant sex-specific difference in Zinc within the patient group with male patients recording higher levels than female patients introduces a nuanced dimension to the Zinc findings that warrants mechanistic discussion (Mirnezami & Rahimi, 2018). The clinical evidence for a significant and generalized serum Zinc deficiency in vitiligo patients has been inconsistent across the literature. A study confirmed this study's borderline finding and noted that serum Zinc concentrations in vitiligo patients were within the reference range and did not correlate with standardized measures of disease extent or activity, concluding that serum Zinc is an insufficiently sensitive surrogate of the intracellular or tissue-level Zinc depletion that may be more functionally relevant to melanocyte biology (Faraj, Kemp & Gawkrödger, 2021; Alshiyab et al., 2019; Golabi et al., 2021). Another study also reported similar findings and proposed that the tight homeostatic regulation of serum Zinc maintained within narrow limits through

hepatic metallothionein-mediated storage and redistribution even during mild systemic depletion renders it poorly responsive as a serum biomarker of cellular Zinc status; erythrocyte Zinc content, platelet Zinc levels, or the activity of Zinc-dependent enzymes such as alkaline phosphatase would constitute more sensitive and biologically informative indicators of functionally relevant Zinc status in vitiligo (Alshiyab et al., 2019). The absence of diagnostic utility for Zinc despite statistically significant group comparisons illustrates the important methodological principle that distributional overlap between groups, even with significantly different means, can prevent reliable individual-level classification (Munoz et al., 2019). The absence of significant correlations between the inflammatory triad and either Vitamin D3 or Zinc within the patient group indicates that Vitamin D3 deficiency and Zinc dysregulation in vitiligo are not secondary consequences of acute-phase inflammatory consumption, but rather arise through independent mechanisms operating in parallel with, but not driven by, the acute-phase inflammatory cascade (Faraj, Kemp & Gawkrödger, 2021). Vitamin D3 deficiency is most plausibly driven by impaired cutaneous synthesis resulting from progressive melanocyte and melanin loss, reduced sun-seeking behavior, or constitutional VDR signalling abnormalities, while Zinc dysregulation appears to reflect nutritional and hormonal factors operating largely independently of the inflammatory network. This mechanistic independence implies that anti-inflammatory treatment strategies targeting the innate immune cascade are unlikely to correct Vitamin D3 deficiency or Zinc dysregulation, and that specific targeted nutritional interventions are needed as complementary components of comprehensive vitiligo management (Alshiyab et al., 2019). The findings of this study establish a coherent, biologically plausible biochemical profile of vitiligo characterized by a profound Vitamin D3 deficiency that simultaneously impairs systemic immune tolerance and melanocyte cytoprotection, and a borderline Zinc reduction that is sex-modulated within the patient group; systematic Vitamin D3 screening and supplementation should be incorporated into the standard management protocol for vitiligo (Rodrigues et al., 2017; Aljbory & Mahmood, 2025).

Conclusion

Vitamin D3 and Zinc levels were markedly lower in patients with vitiligo. This was the most consistent biochemical abnormality and suggested an important role in the disease. Vitamin D3 and zinc did not show significant correlations with inflammatory markers. This suggests that nutritional imbalance and inflammation may run in parallel, not as the same process.

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Ethical approval: Ethical approval for the study was provided by the relevant institutional ethics committee prior to the commencement of the sample collection. (code MIDTUH14 on 16/4/2026).

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