Dermatology Practical & Conceptual

Serum Calprotectin Levels in Vitiligo Patients and Disease Relation

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Key words: Vitiligo, calprotectin, S100A8/A9 protein, oxidative stress, etiology

Citation: Atak T, Kartal SP, Gungor EB, Ucar F. Serum Calprotectin Levels in Vitiligo Patients and Disease Relation. *Dermatol Pract Concept*. 2024;14(3):e2024184. DOI: https://doi.org/10.5826/dpc.1403a184

Accepted: March 22, 2024; **Published:** July 2024

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Funding: None.

Competing Interests: None.

Authorship: All authors have contributed significantly to this publication.

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ABSTRACT

Introduction: Vitiligo is characterized as melanocyte loss in skin and mucous membranes, the pathogenesis of which **has** not yet been clarified. Calprotectin is a protein secreted from neutrophils, monocytes, and dendritic cells which has an effect on cytokine receptor regulation and the production of reactive oxygen radicals. It has been the subject of research in various inflammatory and autoimmune diseases, yet not investigated in vitiligo.

Objective: The aim of **our** study was to investigate the role of calprotectin in the etiopathogenesis of vitiligo and its relationship with clinical subtypes and disease scores.

Methods: Forty-four vitiligo patients with lack of autoimmune disease and 36 age- and sex-matched healthy controls were involved in the study. Serum calprotectin levels were measured by ELISA. The results were **compared** with the control group, and the relationship between patients' demographic characteristics, skin phototypes, disease type, disease scores (Vitiligo Area Scoring Index and Vitiligo Disease Activity Score), disease duration, and age at onset were evaluated.

Results: The median serum calprotectin level was 454.08 pg/ml (41.19–873.41) in the patient group, and the median serum calprotectin level was 223.17 pg/ml (44.88–1044.43) in the control group. Serum **calprotectin** level was significantly higher in the patient group than in the control group ($P = 0.016$). No correlation was found between serum calprotectin level and disease scores, disease duration, age, or age of onset of disease ($P > 0.05$).

Conclusions: In our study, serum calprotectin levels in the patient group were found to be significantly higher than in the control group. Our findings and the existing literature on calprotectin suggest its potential involvement in the pathogenesis of vitiligo, independent of disease progression and patient characteristics.

Introduction

Vitiligo is an acquired skin and mucous disease that occurs as a result of selective loss of melanocytes and is characterized by well-circumscribed depigmented macula or patches. It can be seen at any age and can cause psychological problems due to cosmetic appearance [1-4].

The etiology of vitiligo is unknown. Some of the prevalent hypotheses, including the autoimmune, genetic, oxidative stress, neural, cytotoxic, and metabolic etiologies, have been suggested [5].

A recent study identified key genes that may play a role in pathogenesis. These genes, including S100A8, S100A9, MUC-1, and PPARG, play a role in the intrinsic apoptotic signaling pathway. It is known that epidermal melanocytes are affected by radical oxygen radicals, and it is thought that apoptosis caused by radical oxygen radicals may be responsible for melanocyte loss [6].

Calprotectin is secreted by neutrophils, monocytes, and dendritic cells and consists of a heterodimeric complex of two proteins, S100A8 and S100A9 [7, 8]. When released, it has functions such as damage-associated pattern molecule (DAMP) or function as alarmin and promoting inflammatory response [9]. It have been thought that alarmins are also released in response to cell injury or death. They activate immune cells both in host defence and in disease. S100A8/S100A9, as alarmins, tend to rise after oxidative stress and cell death and are then involved in the aberrant immune response [10]. Calprotectin's effect on cytokine receptor regulation and its role in the production of reactive oxygen radicals are important in chronic inflammatory diseases [8].

Since dendritic cells and cytotoxic T cells are involved in melanocyte destruction in the autoimmune hypothesis in vitiligo pathogenesis, the aim of this study was to examine the role of calprotectin in the pathogenesis of the disease and to determine whether this protein is associated with clinical subtypes and disease scores.

Material and Methods

Forty-four vitiligo patients (age 18-65 years, without inflammatory infectious, other autoimmune diseases or malignancy) admitted to our dermatology outpatient clinic were included in the study. The control group consisted of 36 age- and sex-matched healthy volunteers. The study was conducted in accordance with the Declaration of Helsinki guidelines. All participants were informed about the procedures and their consent was obtained.

Patients were scored according to Vitiligo Area Scoring Index (VASI) [11] and Vitiligo Disease Activity Score (VIDA) [12, 13]. Serum calprotectin levels were compared with the control group, and the relationship between patients' demographic characteristics, skin phototypes, disease type (segmental, generalized, focal, acrofacial, universal), presence of leukotrichia, treatment status in the last six months, disease scores, disease duration, family history, smoking, body mass index, and age at onset were evaluated.

Blood samples were taken in serum separator tubes with clot activator. After waiting for 30 minutes for coagulation, the samples were centrifuged at 1000 x g for 20 minutes and stored at -80 ºC until the day of analysis. Serum calprotectin levels were studied using the Cloud Clone brand (Cloud Clone Corp., USCN Life Science Inc., Houston, TX, USA) (Lot No: L220525461) mass quantitative enzyme-linked immunosorbent assay (ELISA) method. The measuring range of the kit is 31.2-2.000 pg/mL, the sensitivity is 13.6 pg/mL within-run coefficient of variation (CV): <10%, between-study coefficient of variation (CV): <12%.

The obtained data were evaluated in the SPSS (version 15.0) statistical package program. The study presents descriptive data using various statistical measures, including numbers, percentages, means, medians, standard deviations, and minimum-maximum values. The conformity of the data obtained from the scales to the normal distribution was evaluated with the Shapiro-Wilk test. Chi-square test was used to compare categorical data between groups. Mann-Whitney U test, Kruskal-Wallis test, and Spearman's correlation analysis were used in the evaluation of the data after evaluating the conformity to the normal distribution. ROC analysis was performed for the distribution of serum calprotectin values in the both groups.

Results

The mean age (37.38±11.78 and 36.75±9.12 years, respectively) and sex distribution (13 female/31 male and 11 female/25 male, respectively) of vitiligo group and control group were similar. The most common type of vitiligo was the generalized type (70.5%), followed by focal vitiligo (25%) and acrofacial vitiligo (4.5%) (Table 1). The mean disease duration was 4.99±5.39 years and ranged between 0.20 and 26.0 years. The mean ±SD values for VASI and VIDA scores were 3.04±5.66 and 1.59±1.70, respectively.

There was a moderate negative correlation between VASI score and disease onset age (r = -0.414, P = 0.05), and a moderate positive correlation between VASI score and disease duration (r = 0.496, P = 0.01).

The average serum levels of calprotectin in vitiligo patients and controls were 409.11±217.22 and 301.30±229.89, respectively. There was a significant difference between the serum calprotectin levels in patients with vitiligo compared to healthy individuals (P = 0.016) (Table 2).

Fourteen (31.8%) of the patients had at least one family member with vitiligo. Leukotrichia was found in 22 (50%)

Table 1. The Relationship between Patients' Variables and Calprotectin level

		N (%)	Calprotectin level	*P*
Smoking	No	25 (56.8%)	424.19±224.55	0.749
	Yes	19 (43.2%)	363.78±217.76	
Family history	No	30 (68.2%)	430.26±217.38	0.320
	Yes	14 (31.8%)	363.78±217.76	
Leukotrichia	No	22 (50%)	473.45±204.97	0.073
	Yes	22 (50%)	344.77±214.32	
BMI (Body Mass Index)	Normal	20 (45.5%)	441.05±203.01	0.423
	Overweight	24 (54.5%)	382.49±229.23	
Vitiligo subtype	Generalized	31 (70.5%)	423.42±213.60	0.653
	Focal	11 (25%)	356.54±243.51	
	Akrofacial	2 (4.5%)	476.46±146.46	
Skin phototype	2	8 (18.2%)	336.8±192.02	0.671
	3	17 (38. 6%)	405.6±209.41	
	4	18 (40.9%)	437.24±242.01	
	6	1 (2.3%)	540.86	
Treatment (last 6 months)	No	27 (61.4%)	341.66±184.70	**0.013***
	Yes	17 (38.6%)	516.24±226.94	

Table 2. Comparison of Serum Calprotectin Levels of the Patient and Control Groups

	Mean ± SD	Median (min.-max.)	*P* value
Patients	409.11±217.22	454.08 pg/ml (41.19-873.41)	**0.016***
Controls	301.30±229.89	223.17 pg/ml (44.88-1044.43)	

**P < 0.05*

Table 3. Correlation Between Serum Calprotectin Levels and Age, Disease Onset Age, Disease Duration, VASI, and VIDA Scores in Patients

	r	*P**
Age	0.065	0.677
Disease Onset Age	0.117	0.448
Disease Duration	–0.234	0.127
VASI	0.004	0.977
VIDA	–0.161	0.298

**P < 0.05*

of the patients. There was no association between the calprotectin level and family history, leukotrichia, smoking, body mass index, disease subtype, or skin phototype. Seventeen (38.6%) patients had received topical/systemic treatment in the previous six months, five (29.4%) of the patients used topical corticosteroid, and 15 (70.6%) used topical calcineurin inhibitor. Twenty-seven (61.4%) patients had not received any treatment in the previous six months. Serum calprotectin levels were found to be significantly higher in people who had received treatment in the previous six months ($P = 0.013$) (Table 1).

No correlation was found between serum calprotectin level and age, age at disease onset, disease duration, VASI, or VIDA scores in patients (Table 3).

Using the distribution of serum calprotectin values in the patient and control groups, ROC analysis was performed to calculate a cutoff value. In this analysis, sensitivity, specificity, and area under the curve (AUC) were calculated, and the test statistic was significant ($P < 0.001$). The cutoff value was determined as 445.64 pg/ml with the Youden index method (Table 4).

Discussion

The soluble form of calprotectin, which we investigated in our study, can be detected in plasma, body fluids, and feces [7-9]. Serum calprotectin (S100A8/S100A9 protein) has been investigated in inflammatory diseases. Jarlborg et al.'s study of patients with rheumatoid arthritis, axial spondylarthritis, and psoriatic arthritis found that the serum calprotectin levels were higher in all three groups of patients than in the control group [9]. Güzel et al. determined that serum calprotectin levels were higher in patients with psoriasis vulgaris compared to the control group and that they increased with disease severity. The study concluded that calprotectin,

Table 4. Statistical Results of ROC Analysis

Variable	AUC	Standard error	*P* value*	95% Confidence Interval		Cutoff
				Lower Limit	Upper Limit	
Calprotectin	0.657	0.062	0.000	0.535	0.780	445.64

**P < 0.05*

whether released from or found in these cells, might be involved in the pathogenesis [14].

In the literature, calprotectin has also been investigated in autoimmune diseases. In acquired epidermolysis bullosa (EB) and bullous pemphigoid (BP), the MRP 8/14 (calprotectin) protein was observed to be elevated in serum in comparison to the control group. An experimental study was also conducted on mice in same study, and it was stated that calprotectin did not have a role in the effector phase of EB and BP but could potentially have a role in the initial phase [15]. In a study of cutaneous lupus erythematosus, Loser et al. concluded that MRP 8/14 had a crucial role in the development of auto-reactive CD8+ T cells in a model of cutaneous lupus erythematosus [16]. On the other hand, Petersen et al. described the effect of calprotectin on the maturation of dendritic cells in experimental allergic contact dermatitis [17]. Based on the induction of auto-reactive CD8+ T cells by calprotectin in patients with myasthenia gravis, serum calprotectin levels were investigated, with the results showing that the levels in myasthenia gravis patients were higher compared to the control group [18].

In our study, serum calprotectin levels in vitiligo patients were found to be significantly higher than in the control group. In a study investigating S100B protein, which has a DAMP function like calprotectin and is thought to be responsible for cytotoxicity in vitiligo, serum S100B protein was found to be significantly higher in vitiligo patients compared to the control group, and no correlation was found with disease activity or VASI-VIDA scores [19]. In our study, no correlation was found between calprotectin and VASI-VIDA scores. Furthermore, there was no correlation noted between serum calprotectin level and family history, presence of leukotrichia, body mass index, skin phototype, disease type, age, age at onset, or duration of disease. This is probably due to inadequate sample size.

Damage to melanocytes results from an imbalance in oxidative stress according to biochemical hypothesis, and the role of calprotectin in the production of reactive oxygen radicals is known [20]. There is no previous study on calprotectin, which has roles in autoimmunity and oxidative stress mechanisms in vitiligo. However, some limited success has been achieved with antioxidant therapy. In this study, serum calprotectin levels were found to be elevated in people who had received any form of treatment within the previous six months ($P = 0.013$). As vitiligo is a chronic and typically lifelong condition, patients have a tendency to cease treatment with increasing disease duration. Calprotectin levels are expected to be elevated because the patients treated within the last six months are either in early disease or are sufficiently in the active period of the disease to require medical intervention. In other words, since calprotectin also functions as an alarmin whose levels are elevated in cell death and promote inflammation, serum calprotectin levels would be expected to be high in vitiligo patients in the active phase.

The study is limited by its small sample size, the use of only one research method (i.e., ELISA), and the limited number of patients in the vitiligo subgroups.

Conclusion

Our findings and the existing literature on calprotectin suggest its potential involvement in the pathogenesis of vitiligo, independent of disease progression and patient characteristics. However, further investigation is required to determine its impact on autoimmunity and potential oxidative effects on the progression of the disease. This study is expected to facilitate further research, thereby contributing to the understanding of the causes and development of vitiligo, and the creation of new therapeutic agents.

References

1. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. *J Am Acad Dermatol*. 2011;65(3):473-91.
2. Bergqvist C, Ezzedine K. Vitiligo: A Review. *Dermatology*. 2020; 236(6):571-92.
3. Lapeere H, Boone B, De Schepper S, et al. Chapter 75. Hypomelanoses and Hypermelanoses. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K. eds. *Fitzpatrick's Dermatology in General Medicine, 8e*. The McGraw-Hill Companies; 2012.:804-26.
4. Ortonne TPaJ-P. Vitiligo and Other Disorders of Hypopigmentation. In: Jean L. Bolognia Julie V. Schaffer, Lorenzo Cerroni, editors. *Dermatology 4e*. Elsevier;2018:1087-114.
5. Marchioro HZ, Silva de Castro CC, Fava VM, Sakiyama PH, Dellatorre G, Miot HA. Update on the pathogenesis of vitiligo. *An Bras Dermatol*. 2022;97(4):478-90.

6. Liu B, Xie Y, Wu Z. Identification of Candidate Genes and Pathways in Nonsegmental Vitiligo Using Integrated Bioinformatics Methods. *Dermatology*. 2021;237(3):464-72.
7. Stríz I, Trebichavský I. Calprotectin - a pleiotropic molecule in acute and chronic inflammation. *Physiol Res*. 2004;53(3):245-53.
8. Jukic A, Bakiri L, Wagner EF, Tilg H, Adolph TE. Calprotectin: from biomarker to biological function. *Gut*. 2021;70(10):1978-88.
9. Jarlborg M, Courvoisier DS, Lamacchia C, et al. Serum calprotectin: a promising biomarker in rheumatoid arthritis and axial spondyloarthritis. *Arthritis Res Ther*. 2020;22(1):105.
10. He K, Wu W, Wang X, et al. Circulatory levels of alarmins in patients with non-segmental vitiligo: Potential biomarkers for disease diagnosis and activity/severity assessment. *Front Immunol*. 2022;13:1069196.
11. Hamzavi I, Jain H, McLean D, Shapiro J, Zeng H, Lui H. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. *Arch Dermatol*. 2004;140(6):677-83.
12. Njoo MD, Das PK, Bos JD, Westerhof W. Association of the Köbner phenomenon with disease activity and therapeutic responsiveness in vitiligo vulgaris. *Arch Dermatol*. 1999;135(4):407-13.
13. Alghamdi KM, Kumar A, Taïeb A, Ezzedine K. Assessment methods for the evaluation of vitiligo. *J Eur Acad Dermatol Venereol*. 2012;26(12):1463-71.
14. Savas Guzel GE, Mustafa Kulac, Eda Celik Guzel, Volkan Kucukyalcin, Sule Kaya, Ali Riza Kiziler. Chemerin and calprotectin levels correlate with disease activity and inflammation markers in psoriasis vulgaris. *Dermatologica Sinica*. 2015;33(1):1-4.
15. Akbarzadeh R, Yu X, Vogl T, et al. Myeloid-related proteins-8 and -14 are expressed but dispensable in the pathogenesis of experimental epidermolysis bullosa acquisita and bullous pemphigoid. *J Dermatol Sci*. 2016;81(3):165-72.
16. Loser K, Vogl T, Voskort M, et al. The Toll-like receptor 4 ligands Mrp8 and Mrp14 are crucial in the development of autoreactive CD8+ T cells. *Nat Med*. 2010;16(6):713-7.
17. Petersen B, Wolf M, Austermann J, van Lent P, Foell D, Ahlmann M, et al. The alarmin Mrp8/14 as regulator of the adaptive immune response during allergic contact dermatitis. *Embo J*. 2013;32(1):100-11.
18. Stascheit F, Hotter B, Hoffmann S, et al. Calprotectin as potential novel biomarker in myasthenia gravis. *J Transl Autoimmun*. 2021;4:100111.
19. Badran AY, Gomaa AS, El-Mahdy RI, El Zohne RA, Kamal DT, Abou-Taleb DAE. Serum level of S100B in vitiligo patients: Is it a marker of disease activity? *Australas J Dermatol*. 2021;62(1):e67-e72.
20. Kubelis-López DE, Zapata-Salazar NA, Said-Fernández SL, et al. Updates and new medical treatments for vitiligo (Review). *Exp Ther Med*. 2021;22(2):797.