Assessment of tissue level of interleukin-9 in psoriasis and vitiligo

Sara B. Mahmoud[a], Nesma A.M. Salem[b], Olfat G. Shaker[c], Aya M. Fahim[a]

[a]Dermatology Department, Faculty of Medicine, Cairo University, Cairo, Egypt, [b]Dermatology Department, Nasser Institute, Cairo, Egypt, [c]Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

Correspondence to Aya M. Fahim, MD, Faculty of Medicine, Cairo University, PO Box 109, El-Malek El-Saleh, Cairo 11562, Egypt.
Tel: +20 102 027 9628; fax: +20223644383;
e-mail: ayafahim2011@cu.edu.eg

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Background
Psoriasis is a chronic, inflammatory, T-cell-mediated autoimmune disease. Vitiligo is an acquired depigmentary disease that occurs due to the loss of functional melanocytes from the epidermis. Interleukin (IL)-9 is a T cell-derived cytokine that was initially designated as a T helper2 cytokine. There is a link between the expression and action of IL-9 and pro-inflammatory cytokines tumor necrosis factor, IL-1, IL-17, and interferon-γ, suggesting that IL-9 is associated with the pathogenesis of autoimmune diseases.
Objective
To evaluate the tissue levels of IL-9 in patients with psoriasis and vitiligo in comparison with controls, to assess the possible role of IL-9 in the pathogenesis of these diseases.
Patients and methods
This case–control study included 30 patients with psoriasis, 30 patients with vitiligo, and 30 age-matched and sex-matched healthy controls. A skin biopsy was taken from all participants for evaluation of tissue IL-9 levels by enzyme-linked immunosorbent assay.
Results
Tissue IL-9 was significantly higher in patients with psoriasis (28.65±18.456) and patients with vitiligo (51.056±41.536) than controls (P=0.013 and P<0.001, respectively). In addition, it was significantly higher in patients with vitiligo than in patients with psoriasis (P=0.004).
Conclusion
This study suggests a possible role for IL-9 in the pathogenesis of psoriasis and vitiligo by documenting significantly higher tissue levels in patients than in controls.

Keywords:
interleukin-9, psoriasis, vitiligo

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Introduction

Psoriasis is a common chronic immunological skin disease [1]. Pathogenesis is multifactorial, with complex interactions between innate and acquired immune responses [2]. It is believed that psoriasis is mostly a T helper (Th)1/Th17-induced inflammatory disease [3].

Vitiligo is a chronic acquired disease due to loss of melanocytes from the basal epidermal layer [4]. The exact etiology is not well known, and multiple theories have been suggested, including the autoimmune, oxidative stress, and neuroendocrine theories [5].

Interleukin (IL)-9 is one of the members of the IL-2 cytokine family produced by naïve CD4+ T cells in response to transforming growth factor-β and IL-4 (Th9 pathway) [6]. In addition, a number of CD4 T-cell subsets (Th1, Th2, or Th17 cells) can secrete IL-9 in mice [7,8]. IL-9 can stimulate the differentiation of Th17 cells, which mediate several autoimmune and inflammatory diseases [6].

The aim of this study was to evaluate the tissue levels of IL-9 in patients with psoriasis and patients with vitiligo in comparison with controls to highlight the previously suggested role of IL-9 in psoriasis and investigate its possible role in vitiligo.

Patients and methods

30 patients with psoriasis vulgaris, 30 patients with nonsegmental vitiligo, and 30 healthy add were enrolled in this case control study. They were recruited from the dermatology outpatient clinic, Faculty of Medicine, Cairo University, after approval of the Research Ethics Committee, with approval number MS-5-2019. Written informed consent were signed by all participants. This study was conducted during the time interval from June 2019 to December 2019.

Both sexes and patients with age above 18 years old were included. Exclusion criteria were patients with segmental vitiligo, patients with pustular or erythrodermic psoriasis, patients with other autoimmune diseases, patients receiving topical treatment during the past 4 weeks, and patients receiving phototherapy or systemic treatment during the past 12 weeks.

Full history was taken. The dermatological examination was performed, including the skin type, the distribution, the clinical variant of psoriasis (plaque, scalp, nail, flexural, and palmoplantar) or vitiligo (vulgaris, focal, acral, or acrofacial), extent of the disease using the rule of nine in addition to the assessment of vitiligo area and severity index (VASI score) and vitiligo disease activity (VIDA score) for patients with vitiligo [9], as well as psoriatic area and severity index (PASI) score for patients with psoriasis [10]. A punch skin biopsy (4 mm in diameter) was taken from the patients' lesional skin, as well as controls, and frozen at -80°C for assessment of tissue levels of IL-9 by enzyme-linked immunosorbent assay (ELISA).

Laboratory methods

Quantitation of interleukin-9 in skin biopsy

Each skin biopsy was weighed and homogenized in PBS using a homogenizer. The homogenate was centrifuged, and the supernatant was separated for determination of IL-9 by the ELISA technique. ELISA kit was supplied by Sun Long Biotech Co. Ltd in Hangzhou, Zhejiang (China) Catalog Number: SL1005Hu. This ELISA kit uses sandwich-ELISA as the method. The concentration of human IL-9 in the sample was detected from the standard curve.

Statistical analysis

Data were statistically described in terms of mean±SD, median and range, or frequencies and percentages. Comparison was done using Mann–Whitney *U* test for non-normal data. Comparison between more than two groups was done using one-way analysis of variance test with post-hoc multiple two-group comparisons. For comparing categorical data, χ^2 test was performed, or the exact test when the expected frequency was < five. Correlations were done using Pearson and Spearman rank correlation equations for normally and non-normally distributed variables, respectively. *P* values <0.05 were considered statistically significant. All statistical calculations were done using the computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp., Armonk, New York, USA) release 22 for Microsoft Windows.

Results

Demographic, clinical, and laboratory data of the three studied groups are presented in Table 1.

There was no statistically significant difference among the three groups regarding age or sex (P=0.589 and 0.585, respectively). Tissue IL-9 was significantly higher in patients with psoriasis and patients with vitiligo patients than in controls, with a significant difference between patients with vitiligo and controls (P=0.013 and P<0.001, respectively). In addition, it was significantly higher in patients with vitiligo than in patients with psoriasis (P=0.004) (Fig. 1).

There was no statistically significant difference between males and females regarding tissue IL-9 in either patients with psoriasis or patients with vitiligo (P=0.138 and 0.787, respectively). There was no statistically significant difference between patients with stable (n=5, 16.7%) and active vitiligo (n=25, 83.3%) regarding tissue IL-9 (P=0.388). There was no significant difference between vitiligo vulgaris (n=27, 90%) and focal vitiligo (n=3, 10%) regarding tissue IL-9 levels (P=0.351).

In the psoriasis group, there was no statistically significant correlation between tissue IL-9 and either age, duration of disease, extent, or PASI score (r=0.202, P=0.284; r=0.085, P=0.653; r=0.137, P=0.469; and r=0.125, P=0.511, respectively). In the vitiligo group, there was no statistically significant correlation between tissue IL-9 and either age, duration of disease, extent, VIDA, or VASI scores (r=-0.169, P=0.373; r=0.005, P=0.979; r=0.302, P=0.105; r=0.035, P=0.853; and r=0.311, P=0.094, respectively) (Tables 2 and 3).

Discussion

This study detected significantly higher tissue IL-9 levels in patients with psoriasis than in controls. Supporting our findings, a previous study reported increased tissue expression of IL-9 and IL-9R in mice with psoriasis-like phenotype. In addition, IL-9R expression was significantly higher in the lesional skin of patients with psoriasis than in controls [6]. Another study reported that IL-9-producing T cells were evident in the skin lesions of patients with psoriasis and patients with atopic dermatitis (AD), and their number was detected to be higher significantly in psoriatic skin lesions compared with controls [11].

Regarding serum IL-9 levels, Cardoso *et al.* [12] detected significantly higher serum IL-9 in psoriasis

Table 1 Clinical and laboratory data of the studied groups

Variables	Psoriasis patients (*N*=30)	Vitiligo patients (*N*=30)	Control group (*N*=30)	*P* value
Age (years)				
Range	18–67	18–66	18–66	0.589†
Mean±SD	37.70±14.974	36.30±15.327	40.30±15.430	
Sex [*n* (%)]				
Males	18 (60)	14 (46.7)	16 (53.3)	0.585‡
Females	12 (40)	16 (53.3)	14 (46.7)	
Family history [*n* (%)]				
No	22 (73.3)	23 (76.7)		
Yes	8 (26.7)	7 (23.3)		
Duration (months)				
Range	3–404	3–312		
Mean±SD	87.43±86.182	78.43±75.207		
VIDA				
Range		0–4		
Median		3		
Activity [*n* (%)]				
Stable		5 (16.7)		
Active		25 (83.3)		
Type [*n* (%)]				
Plaque psoriasis	30 (100)			
Scalp psoriasis	22 (73.3)			
Nail psoriasis	6 (20)			
Flexural psoriasis	6 (20)			
Palmoplantar psoriasis	6 (20)	27 (90)		
Vitiligo vulgaris		3 (10)		
Focal vitiligo				
Extent (%)				
Median (range)	10 (2–50)	3.25 (1–75)		
Mean±SD	14.07±12.387	11.67±19.048		
PASI				
Median (range)	6.450 (2–15.3)			
Mean±SD	7.317±3.819			
VASI				
Median (range)		2.825 (0.9–75)		
Mean±SD		10.96±18.578		
Tissue IL-9 (ng/mg)				
Median (range)	23.206 (11.4–199.5)	35.151 (5.9–13.0)	8.535 (11–87.5)	<0.001†*
Mean±SD	28.65±18.456	51.056±41.536	8.765±1.913	

IL-9, interleukin-9; PASI, psoriatic area and severity index; VASI, vitiligo area and severity index; VIDA, vitiligo disease activity. †Analysis of variance test. ‡χ^2 test. *P value <0.05 is considered statistically significant.

Table 2 Comparison between males and females regarding tissue interleukin-9

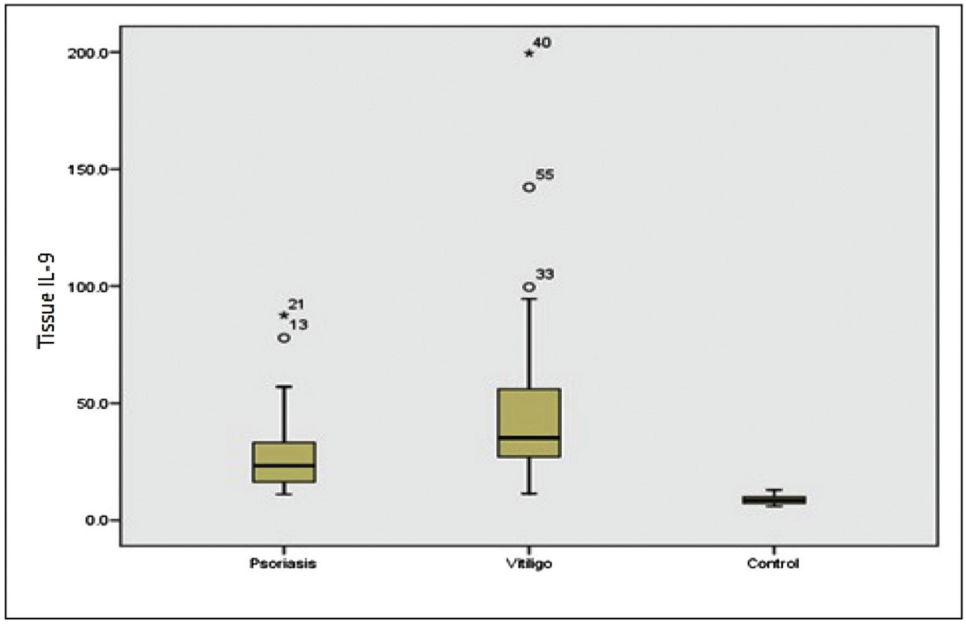
Tissue IL-9 (ng/mg)	Males	Females	*P* value
Psoriasis			0.138
Range	11–87.50	14.7–57	
Mean±SD	26.968±21.316	31.172±13.574	
Vitiligo			0.787
Range	17.6–142.3	11.4–199.5	
Mean±SD	48.672±42.893	51.781±42.072	

IL-9, interleukin-9.

patients compared with controls. Also, Hamza *et al.* [13] detected significant higher gene expression levels of serum IL-9 in patients with AD and patients with psoriasis patients than in controls. In the opposite side, the study performed by Ma *et al.* [14] detected no significant difference between patients with psoriasis and controls regarding serum IL-9 levels. Both studies focused on patients with AD and enrolled patients with psoriasis as controls.

The role of IL-9 in psoriasis could be explained by its pleiotropic activities. IL-9 acts as a growth factor for T cells and mast cells, which secrete pro-inflammatory and pro-angiogenic factors, including IL-8, IL-17, tumor necrosis factor, hepatocyte growth factor, fibroblast growth factor-2, and vascular endothelial growth factor [15,16]. Supporting this role, Singh

Figure 1



Comparison among the three studied groups regarding tissue IL-9. Tissue IL-9 was significantly higher in patients with psoriasis and vitiligo than in controls (P=0.013 and P<0.001, respectively). In addition, it was significantly higher in patients with vitiligo than in patients with psoriasis (P=0.004). IL-9, interleukin-9.

Table 3 Comparison between patients with stable and active vitiligo, as well as vitiligo vulgaris and focal vitiligo regarding tissue interleukin-9

Tissue IL-9 (ng/mg)	Vitiligo vulgaris	Focal vitiligo	*P* value	Stable	Active	*P* value
Range	11.4–199.5	16.1–55.1	0.351	30.1–81.1	11.4–199.5	0.388
Mean±SD	53.055±43.036	33.066±19.965		49.4±19.8	51.4±44.9	

IL-9, interleukin-9.

et al. [6] demonstrated that the injection of IL-9 into the skin of mice with psoriasis-like phenotype stimulated hyperplasia of the epidermis and skin infiltration by T cells, mast cells, and CD68+ monocytes/macrophages and induced angiogenesis and overexpression of vascular endothelial growth factor and CD31. These actions were reversed by anti-IL-9 antibodies injection in mice in vivo. In addition, IL-9 increased tubal formation by human endothelial cells *in vitro* [6].

Moreover, a link has been found between IL-9 and the Th17 pathway, which plays an important role in the pathogenesis of psoriasis [6]. IL-9 receptors were expressed by TH17 cells [17], which can contribute directly to their differentiation [18]. Intradermal injection of IL-9 in transgenic mice induced Th-17-mediated skin inflammation, by the expression of IL-17A. Furthermore, IL-9 significantly induced IL-17A secretion by cultured human peripheral blood mononuclear cells and CD4+ T cells of psoriasis patients [6]. In addition, anti-IL-9 treatment decreased the mRNA expression of IL-17A and STAT3. On the contrary, injection of anti-IL-17 antibody in mice inhibited the psoriatic-like skin phenotype and downregulated IL-9 mRNA in the skin and protein levels in serum. Together, these data revealed a positive feedback loop between IL-9 and IL-17A [6].

More evidence concerning the IL-9 role in psoriasis may also be added by the location of the IL-9 gene on chromosome 5 (5q31.1) [18], a psoriasis susceptibility region (5q31.1-q33.1) [19].

However, no significant relationship between tissue IL-9 and either the extent of the lesions or the PASI score was detected in the present study. This could be explained by the relatively small sample size, or IL-9 being not the only player in the complex multifactorial pathogenesis of psoriasis, or acting through indirect mechanisms.

Regarding patients with vitiligo, our study revealed significantly higher tissue IL-9 than in controls. In addition, tissue IL-9 levels were detected to be higher

significantly in patients with vitiligo than in patients with psoriasis.

In this context, Czarnowicki *et al.* [20] demonstrated that IL-9-producing T cells were increased significantly in the blood of vitiligo patients than in controls. In addition, interferon -γ, IL-13, IL-22, and IL-17A-producing cells were significantly higher in the blood of patients with vitiligo in comparison with controls and other autoimmune diseases including psoriasis. The authors concluded that vitiligo is characterized by a systemic nature and greater autoimmune activation compared with other autoimmune diseases, as well as multicytokine polarization [20]. Another study conducted by Kumar *et al.* [21] reported significantly higher numbers of skin-homing and systemic Th9 cells in patients with vitiligo than in controls.

Th9 cells produce IL-9, which is essential for the production of other cytokines, including interferon-γ and IL-17, in addition to co-expression of tumor necrosis factor-α and granzyme B [22]. Moreover, IL-9 enhanced the recruitment of Th17 cells, as well as dendritic cells and cytotoxic T cells [23]. Based on these findings, IL-9-producing cells might play a role in vitiligo pathogenesis.

However, our study revealed no significant relationship between tissue IL-9 and either the extent of the lesions, VIDA, or VASI scores. This could be explained by the relatively small sample size, or IL-9 being not the only player in the complex multifactorial pathogenesis of vitiligo, or acting through indirect mechanisms.

IL-9 provides a potential therapeutic target for the treatment of psoriasis as well as vitiligo, and studies reported several possible anti-IL-9 therapeutic options. MEDI-528, a humanized anti-IL-9 monoclonal antibody, demonstrated efficacy in patients with asthma [24]. In clinical trials, rhIL-9-ETA', a new chimeric toxin formed by fusing hIL-9-cDNA into modified *Pseudomonas aeruginosa* exotoxin A (ETA'), has shown efficacy in human malignancies expressing IL-9 and hIL-9R in humans. A JAK1/JAK2 inhibitor suppressed the Th9 cell signaling pathway and decreased inflammatory cytokine mRNA levels in the draining lymph nodes of mice [25].

Possible limitations of the study were the relatively small sample size and deficiency measuring IL-9 levels in nonlesional skin of patients.

In conclusion, tissue IL-9 was detected to be high significantly higher in patients with psoriasis and patients with vitiligo than in controls. In addition, it was significantly higher in patients with vitiligo than in patients with psoriasis. From the results of this study, we suggest that IL-9 could play a role in the pathogenesis of psoriasis and vitiligo.

Further studies are required with a larger number of participants to assess the serum IL-9 levels, to study the relation between IL-9 and IL-17, and to evaluate IL-9 as a possible therapeutic target in the treatment of both psoriasis and vitiligo.

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Conflicts of interest
There are no conflicts of interest.

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