BENHA MEDICAL JOURNAL

Print ISSN 1110-208X.

Online ISSN 2357-0016

Original article

Dynamin-Related Protein 1 (Drp1) Tissue Expression in Vitiligo Patients

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Received:

Accepted:

Abstract

Background: Vitiligo is a chronic skin condition causing depigmented patches due to melanocyte loss, with psychological and quality-of-life impacts. Mitochondrial dysfunction, particularly involving Drp1, contributes to cellular stress and may play a role in vitiligo. Understanding Drp1's involvement could offer new insights and therapeutic targets for the disease. **Aim:** The current study aimed to assess the tissue expression of Drp1 in vitiligo lesional and perilesional skin. **Patients and methods:** This comparative cross-sectional study was conducted on 42 vitiligo patients at Benha University Hospital. Patients underwent clinical evaluations, including history taking and dermatological examination. Vitiligo Area Scoring Index (VASI), Vitiligo Disease Activity Score (VIDA) and Wood's lamp examination. Immunohistochemical analysis was performed on skin biopsies to assess Drp1 expression, with staining intensity and epidermal layer involvement scored systematically. **Results:** Most vitiligo patients were middle-aged females, with stress as the most common trigger and extremities as the most affected site. DRP-1 levels were significantly higher in lesional skin, with greater extension to all epidermal layers compared to perilesional areas. However, DRP-1 scores showed no significant correlation with clinical features such as age, disease extent, VASI, or VIDA scores **Conclusion:** Drp1 expression is significantly higher in lesional skin of vitiligo patients, suggesting its key role in disease pathogenesis and its potential role as a therapeutic target.

Key words: Drp1, Immunohistochemistry, Lesional skin, Mitochondrial dysfunction, Vitiligo

Introduction:

Vitiligo represents the most prevalent depigmenting disorder of the skin, with its global prevalence estimated to range from 0.5% to 1% of the general population. Clinically, it is characterized by well-defined, milky-white depigmented macules that may appear in various anatomical distributions [(1).]

The pathogenesis of vitiligo is multifactorial, involving the progressive loss of functional melanocytes. Several mechanisms have been proposed to explain melanocyte destruction, including genetic predisposition, autoimmune activity, oxidative stress, inflammatory mediator production, and melanocyte detachment. Both innate and adaptive immune responses are believed to play critical roles in the disease process [(2).]

Various immune cells and cytokines are implicated in vitiligo pathophysiology, among which cytotoxic CD8+ T lymphocytes, specific to melanocytes, are considered pivotal. Inflammatory chemokines, particularly C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10, are thought to mediate the recruitment of melanocyte-specific CD8+ T cells to the basal epidermis, where they contribute to melanocyte destruction and consequent melanin loss [(3).]

Oxidative stress is another key contributor to vitiligo onset. Accumulation of reactive oxygen species (ROS), including hydrogen peroxide, coupled with diminished levels of antioxidative enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, creates a cytotoxic environment that promotes melanocyte damage [(4)].

Dynamins, a group of GTPase enzymes, are involved in intracellular membrane fission events, playing essential roles in processes such as phagocytosis and both caveolae- and clathrin-mediated endocytosis. Among the three classical dynamins, Dynamin 1 is neuron-specific, Dynamin 2 is expressed in all tissues, and Dynamin 3 is localized primarily in the brain, lungs, heart, tests, and blood cells [(5)]**.** Dynamin-related protein 1 (DRP1), a member of this protein family, is particularly involved in mitochondrial fission, as well as the preservation of mitochondrial morphology and division [(6)].

Although DRP1 has been linked to various inflammatory disorders, studies exploring its role in skin pathology remain limited [(7)]

So, the current study aimed to assess the tissue expression of Drp1 in vitiligo lesional and perilesional skin.

Patients and Methods

This research was conducted as a comparative cross-sectional study from December 2022 to May 2023. It was approved by the Local Ethics Committee on Research Involving Human Subjects of Benha Faculty of Medicine (Ms 37-11-2022). 42 patients of vitiligo were randomly selected

from the Outpatient Clinic of Dermatology, Venereology, and Andrology, Benha University Hospital, Benha, Egypt. Informed consent was given by all the patients after being well informed regarding the nature of the study and the procedures.

The subjects were males and females of various ages with various patterns and severities of vitiligo. Exclusion criteria were pregnant or lactating women, patients under 18 years of age, and patients with immunosuppression, HIV, or chronic conditions such as renal failure, hepatic insufficiency, cardiovascular diseases, uncontrolled diabetes, or thyroid disease. Patients with autoimmune diseases, such as autoimmune thyroiditis, were also excluded. Moreover, patients who had been using topical treatment for vitiligo during the last month or systemic treatment for the last three months prior to recruitment were not included in the study.

All patients underwent a thorough clinical assessment. A detailed history was taken using a pre-designed sheet, which included personal information (name, age, sex, occupation, smoking habits), disease history (onset, duration, course, and precipitating factors like stress), drug history (including treatments for vitiligo or other systemic diseases), and family history of vitiligo.

A general examination was performed to rule out any systemic diseases unrelated to vitiligo. Local dermatological examination included visual clinical diagnosis of vitiligo, characterized by well-defined, depigmented patches surrounded by normal skin. Patients were classified into two main groups: localized and generalized vitiligo. Localized forms included segmental (affecting one or more body segments without crossing the midline), focal (localized macules with undefined segmental distribution), and mucosal types (affecting mucous membranes). Generalized types included vulgaris (widely scattered patches), acrofacial (affecting distal extremities and face), and universal (complete or near-complete depigmentation).

Regarding diagnostic Tools: Wood's light examination was conducted in a dark room, where lesions appeared bright bluish white, enhancing contrast between affected and unaffected skin and aiding in margin definition. Surface area involvement was measured using the hand unit method, with one hand (palm and fingers) representing approximately 1% of total body surface area.

Disease severity was assessed using the Vitiligo Area Scoring Index (VASI), calculated by multiplying the percentage of involvement in six body regions by residual depigmentation levels (ranging from 0% to 100%) [(8)].

Vitiligo activity was evaluated using the Vitiligo Disease Activity Score (VIDA), a six-point scale based on the patient's perception of lesion progression or appearance of new lesions. Scores ranged from +4 (highly active disease, within 6 weeks) to -1

(stable with spontaneous repigmentation for over a year) [9].

Immunohistochemical (IHC) Analysis was performed on two punch biopsies (one lesional, one perilesional) were taken from each patient. Samples were fixed in 10% formalin and processed at the pathology department of Benha University. Sections were cut into 5-µm slices, deparaffinized in xylene, dehydrated in graded alcohol, and stained with hematoxylin and eosin (H&E) for histological examination and with Drp1 immunostain for protein expression analysis.

Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Sections were washed in PBS, then incubated overnight at 4°C with monoclonal anti-Drp1 antibody (Abcam, USA). Super Picture Polymer Detection Kit (Thermo Fisher Scientific, China) was used for HRP polymer conjugation and DAB chromogen visualization, and hematoxylin counterstaining.

Drp1 expression was scored based on the extent of epidermal staining (1 = basal layer, 2 = middle layers, 3 = all layers) and intensity (0 = none, 1 = weak, 2 = moderate, 3 = deep), with a total score of 1 to 6.

Approval code: (Ms 37-11-2022).

Statistical Analysis:

All data handling and statistical analyses were conducted using IBM SPSS Statistics version 28 (Armonk, NY, USA). The distribution of quantitative variables was assessed for normality employing the Shapiro-Wilk test, complemented by visual inspection techniques. Based on the outcome of these assessments, quantitative data were summarized either as mean values with standard deviations or as medians accompanied by their respective ranges. Categorical data were described using frequencies and corresponding percentages. To compare the intensity and extent of DRP-1 expression between lesional and perilesional skin areas, the sign test was utilized. The Wilcoxon signed-rank test was employed to evaluate differences in the total DRP-1 score between these two regions. Associations between total DRP-1 expression and various clinical parameters were explored using Spearman's rank correlation coefficient. Furthermore, comparisons of total DRP-1 levels across categorical subgroups were performed using the Mann-Whitney U test. All statistical procedures were two-tailed, with a p-value of <0.05 considered indicative of statistical significance.

Results:

The studied patients had a mean age of 47 ±17 years. There was a female predominance (78.6%). A minority of the patients were smokers (7.1%). **Table 1**

The disease trajectory revealed that the onset was gradual in most patients (92.9%), with a mean age of onset of 43 ±17 years. Lesions were most located on the extremities (64.3%), followed by the trunk (21.4%), and the

face and neck (14.3%). A progressive course was the most frequently observed pattern (71.4%), while intermittent progression was seen in 14.3% of patients. Regressive and stationary courses were each reported in 7.1% of cases. **Table 2**

There was a predominance of non-segmental distribution among patients (71.4%), followed by segmental distribution (14.3%), with acrofacial and mixed patterns each observed in 7.1% of cases. The median surface area affected was 2.8%, ranging from 1% to 86%. The median VASI and VIDA scores were 2.4 and 4, respectively, with VASI scores ranging from 1 to 85.8 and VIDA scores ranging from -1 to 4. **Table 3**

Histological analysis revealed reduced melanocyte count and melanin in lesional skin, with mild perivascular lymphocytic infiltration, while perilesional areas showed slightly increased melanocytes and melanin pigment. **Figure 1**

Immunostaining of DRP-1 showed focal nuclear expression in the basal layer of perilesional skin and moderate nuclear expression across all epidermal layers in lesional skin. **Figure 2**

The DRP-1 immunohistochemical staining intensity did not significantly differ between lesional and perilesional areas (P = 0.219). **Table 4**

The DRP-1 level significantly differed between lesional and perilesional areas (P = 0.006), with DRP-1 extension to basal layer being higher in perilesional than lesional areas (85.7 vs. 14.3%). Additionally, extension to all layers was higher in lesional than in perilesional areas (28.6% vs. 7.1%). **Table 5, Figure 3**

DRP-1 score was significantly higher in lesional than perilesional skin (4 vs. 3, respectively, P = 0.007). **Table 6**

Table 1: Demographics of the studied patients

Demographics		
Age (years)	Mean ±SD	47 ±17
Gender		
Males	n (%)	9 (21.4)
Females	n (%)	33 (78.6)
Smoking	n (%)	3 (7.1)
Working status	n (%)	18 (42.9)

SD: Standard deviation.

Table 2: Disease trajectory in the studied patients

	n (%)
Onset	
Gradual	39 (92.9)
Sudden	3 (7.1)
Age of onset (years)	43 ±17
Site of lesion	
Extremity	27 (64.3)
Face & neck	6 (14.3)
Trunk	9 (21.4)
Course	
Intermittent	6 (14.3)
Progressive	30 (71.4)
Regressive	3 (7.1)
Stationary	3 (7.1)

Table 3: Examination findings in the studied patients

Examination findings		
Distribution		
Acrofacial	n (%)	3 (7.1)
Mixed	n (%)	3 (7.1)
Non segmental	n (%)	30 (71.4)
Segmental	n (%)	6 (14.3)
Surface area affected (%)	Median (range)	2.8 (1 - 86)
VASI score	Median (range)	2.4 (1 - 85.8)
VIDA score	Median (range)	4 (-1 - 4)

VASI: Vitiligo Area Scoring Index; VIDA: Vitiligo Disease Activity.

Table 4: DRP-1 intensity in lesional and perilesional areas

DRP-1 intensity	**n (%)**	**P-value**
Lesional area		0.219
No staining	0 (0)	
Week	6 (14.3)	
Moderate	27 (64.3)	
Deep	9 (21.4)	
Perilesional		
No staining	0 (0)	
Week	12 (28.6)	
Moderate	30 (71.4)	
Deep	0 (0)	

Table 5: DRP-1 level of extension in lesional and perilesional areas

DRP-1 level of extension	n (%)	P-value
Lesional		**0.006***
Basal layer	6 (14.3)	
Middle layer	24 (57.1)	
All layers	12 (28.6)	
Perilesional		
Basal layer	36 (85.7)	
Middle layer	3 (7.1)	
All layers	3 (7.1)	

*Significant P-value, n: number, DRP: Dynamin-Related Protein.

Table 6: DRP-1 score in lesional and perilesional areas

DRP-1 total score	Median (range)	P-value
Lesional	4 (2 - 6)	**0.007***
Perilesional	3 (2 - 5)	

***Significant P-value, DRP: Dynamin-Related Protein.

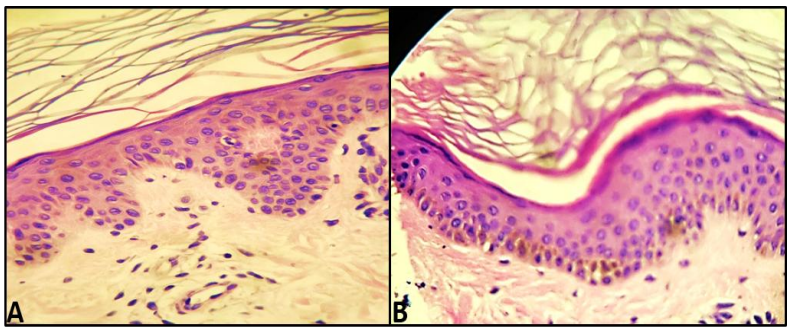

Figure 1: (A) Section in lesional skin showed decreased melanocytes and melanin pigment in basal layer of the epidermis. The dermis showed mild perivascular inflammatory infiltrate mainly lymphocytes, H&E×400. (B) Section in perilesional skin showed slightly increased melanocytes and melanin pigment in basal cell layer of the epidermis, H&E×200.

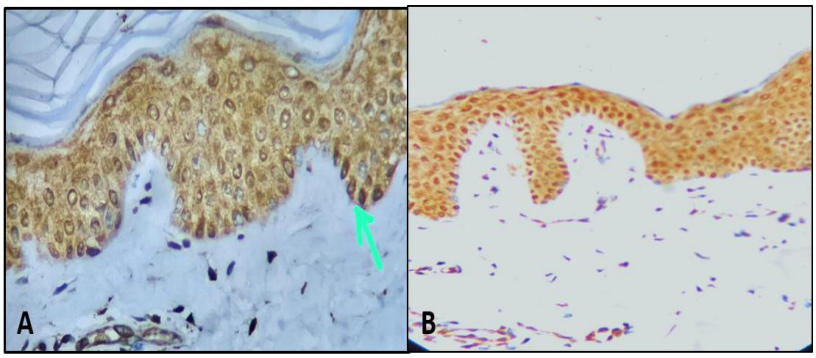

Figure 2: (A) Section in perilesional skin showed focal nuclear dynamin expression in basal cell layer of moderate intensity, ABC×400. (B): Section in lesional skin showed nuclear dynamin expression of moderate intensity in all epidermal layers, ABC×200.

Discussion

Vitiligo is a complex depigmenting disorder marked by melanocyte loss due to genetic, autoimmune, and oxidative factors, with recent studies suggesting mitochondrial dysfunction involving Dynamin-Related Protein 1 (DRP1) may play a role [10]. The current cross-sectional study was conducted on 42 vitiligo patients to assess DRP1 expression in lesional and perilesional skin, indicated its potential involvement in vitiligo pathogenesis.

Regarding demographic data of the studied patients, the studied patients had a mean age of 47 ±17 years. There was a female predominance (78.6%). A minority of the patients were smokers (7.1%).

In harmony, a study conducted to assess the demographic profile and clinical characteristics of vitiligo patients, involving a cohort of 115 individuals recruited from outpatient visits to the Dermatovenereology Department at General Hospital Surabaya, indicated a slight female predominance, with 53.9% of the participants being women [11].

In the current work, regarding triggering factors, our results revealed that the most common triggering factor was stress (50%), followed by sun exposure (35.7%) and trauma (21.4%).

Furthermore, the clinical data demonstrated that most patients (92.9%) experienced a gradual onset of vitiligo, with the mean age of onset being 43 ± 17 years.

This gradual manifestation aligns with the understanding that vitiligo develops over time, often without immediate or obvious triggers. The slow progression may be due to the gradual loss of melanocytes, the cells responsible for producing skin pigment, through autoimmune mechanisms. The age of onset suggests a potential link between vitiligo and mid-life immune system changes, although it can occur at any age. The preference for lesions to appear first on the extremities (64.3%), followed by the trunk (21.4%), and then the face and neck (14.3%) may reflect variations in skin exposure, immune activity, or melanocyte resilience across different body regions [12].

The course of vitiligo was predominantly progressive in 71.4% of the patients, indicating that for the majority, the condition worsened over time, either through the enlargement of existing patches or the development of new ones. An intermittent course, observed in 14.3% of patients, suggests periods of stability interspersed with episodes of activity, possibly triggered by external or internal factors like stress, skin trauma, or systemic changes. The progressive nature of most cases highlights the chronic and often unpredictable evolution of vitiligo, revealing the need for ongoing management strategies to control its spread and mitigate its psychological and cosmetic impact [13].

Consistent with the findings of the current study indicating a gradual progression of vitiligo in most patients, ***Hamzavi et al.,2023*** [14] conducted a

large-scale global survey involving 3,541 adult individuals (≥18 years) diagnosed with vitiligo to investigate its natural course and management from the patient perspective. Their results showed that a significant proportion of participants reported either slow (36.0%) or rapid (31.9%) disease progression, while 8.4% experienced no progression following the initial appearance of lesions. The average time between first noticing skin changes and receiving a formal diagnosis was reported to be 2.4 ± 4.1 years. Anatomically, lesions first appeared on the hands (40.0%) and face (38.6%). Additionally, more than half of the respondents (57.5%) indicated bilateral involvement of the disease [(14)]

Regarding examination findings, the present study revealed predominance of non-segmental distribution (71.4%), followed by segmental (14.3%), with equal acrofacial and mixed distribution (7.1% for each). The median surface area affected was 2.8%, ranging from 1 – 86%. In addition, VASI and VIDA scores had median values of 2.4 and 4, respectively. The VASI score ranged from 1 – 85.8, while VIDA score ranged from -1 – 4.

Similarly, in the study by ***Saiboo et al., 2023***[(11)] non-segmental vitiligo (53.04%) was reported to be more common than segmental vitiligo (46.96%) [(11).]

Also, regarding the affected surface area, ***Hamzavi et al.,2023***[(14)] found that the median surface area affected was 4%, ranging between (0–73.9 %) [(14)].

Regarding DRP-1 intensity in lesional and perilesional areas, our results detected no significant difference in DRP-1 intensity between lesional and perilesional areas (P = 0.219). Concerning the DRP-1 level of extension, the DRP-1 level significantly differed between lesional and perilesional areas (P = 0.006), DRP-1 expression was more limited to the basal layer in perilesional than in lesional areas (85.7 vs. 14.3%), and its extension to all epidermal layers was higher in lesional than in peri-lesional areas (28.6% vs. 7.1%). DRP-1 score was significantly higher in lesional than perilesional skin (4 vs. 3, respectively, P = 0.007) [(14)].

The observation that the Dynamin-related protein 1 (Drp1) total score was significantly higher in lesional than perilesional skin in vitiligo patients points to the critical role of mitochondrial dynamics in the pathogenisis of the disease as Drp1 is a crucial regulator of mitochondrial fission.

In a previous study done on psoriasis by ***Weng et al., 2020*** [(7)], punch skin biopsies were collected from lesional skin of psoriasis vulgaris patients (n = 50) as well as from age and sex-matched healthy controls (n = 50). They found that in normal control skin, Drp1 was sporadically expressed in the basal layer, whereas in psoriasis vulgaris lesions, Drp1 protein was expressed throughout the basal and suprabasal layers. The IHC score for Drp1 immunostaining further showed that Drp1 protein was significantly upregulated in psoriatic lesional skin.

Drp1 is instrumental in mitochondrial fission, a process that, when dysregulated, can lead to mitochondrial dysfunction and increased oxidative stress [7].

Accordingly in vitiligo, the same mechanism may contribute to the destruction of melanocytes, the pigment-producing cells whose loss is characteristic of the disease. Elevated Drp1 levels in lesional areas suggest that mitochondrial fission is more active here, potentially as a response to cellular stress induced by the disease process, leading to an imbalance that favors cell damage and death [15]

On the other hand, this increase in Drp1 activity might reflect the skin's attempt to cope with the pathological environment by isolating damaged mitochondria and thus mitigating further cellular harm. The interplay between Drp1-mediated mitochondrial dynamics and immune responses, especially in lesional skin where melanocyte destruction is actively ongoing, could influence the progression of vitiligo [16].

Elevated Drp1 could also be involved in tissue remodeling and repair mechanisms in response to the disease. Hence, understanding the contributions of Drp1 and mitochondrial dynamics to vitiligo pathology shows promising avenues for developing targeted therapies that address the mitochondrial dysfunction and oxidative stress central to the disease [17].

Our study lays the groundwork for a deeper understanding of vitiligo at the molecular level, opening new avenues for research into the mechanisms underlying this complex disorder. By pinpointing Drp1 as a key player in the disease's pathology, we provide a novel target for therapeutic intervention, which could lead to more effective treatments for patients suffering from vitiligo as it’s the first study performed to assess the tissue expression of DRP-1 in vitiligo lesional and perilesional skin.

Conclusion

In this study, we found that Dynamin-related protein 1 (Drp1) expression significantly differs between lesional and perilesional skin in vitiligo patients, with higher Drp1 levels and more extension of staining to all epidermal layers observed in lesional areas. These findings show that Drp1 may play a critical role in the pathogenesis of vitiligo, potentially offering a new target for therapeutic

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To cite this article: Asmaa S. Abd Elmoneim, Menha A. Ibrahim, Shaimaa K.Abdelwahab, Aml Y. Habashy. Dynamin-Related Protein 1 (Drp1) Tissue Expression in Vitiligo Patients. BMFJ XXX, DOI: 10.21608/bmfj.2025.387639.2428