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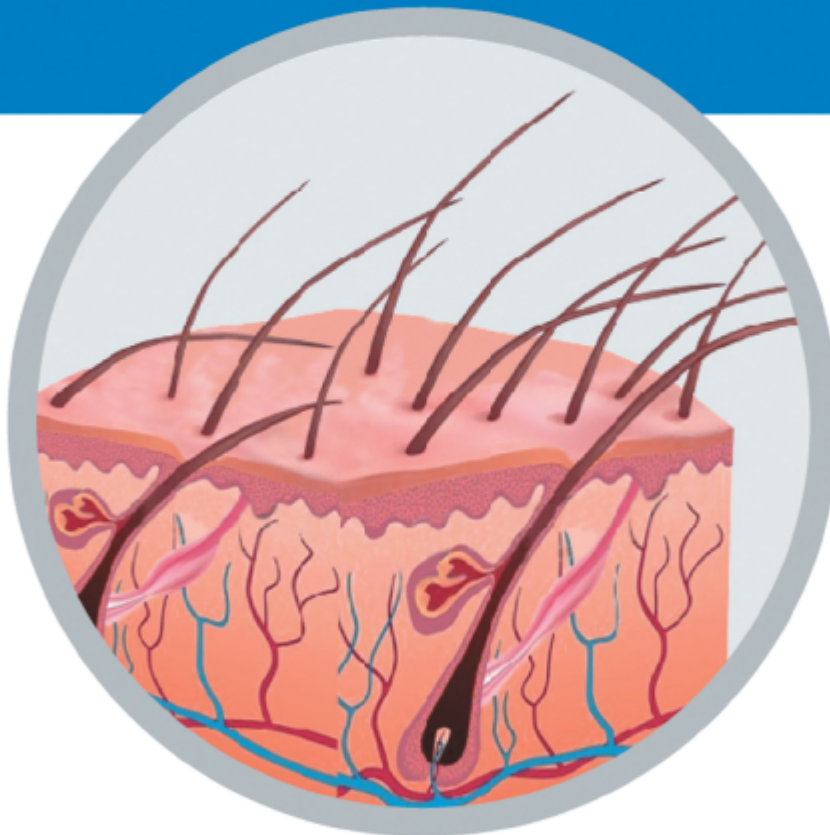
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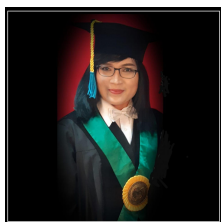
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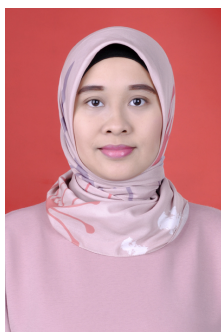
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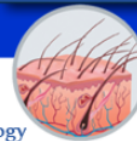
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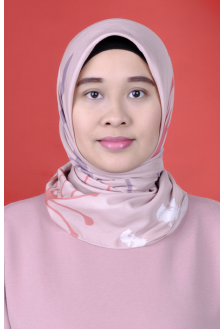
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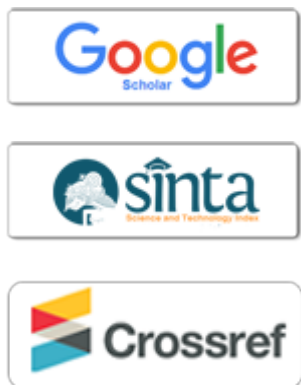
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Expression of Melan-A in Depigmented Skin of Vitiligo Patients

Tjokorde Istri Nindya Vaniary, Muhammad Yulianto Listiawan, Dwi Murtiastutik

Departement of Dermatology and Venereology

Faculty of Medicine Universitas Airlangga/Dr. Soetomo General Academic Teaching Hospital Surabaya

ABSTRACT

Background: Vitiligo is an acquired and commonly found pigmentation disorder characterized by milky-white patches on the skin, hair, and mucosa due to melanocyte damage. The cause of vitiligo is still unclear. A study proves that cell-mediated immunity plays a role in the pathogenesis of vitiligo. Melan-A is a melanoma-related antigen that is recognized by autologous cytotoxic T cells and one of the critical markers for detecting melanocytes. **Objective:** To evaluate the expression of Melan-A in depigmented lesions of vitiligo patients. **Methods:** A descriptive study aimed to describe the expression of Melan-A in the depigmented skin of vitiligo patients at the Dermatovenerology Outpatient Clinic Cosmetic Division of Academic General Hospital Dr. Soetomo Surabaya. Eleven study subjects were selected through a sequence of selection. **Results:** Melan-A expression in the depigmented skin of vitiligo patients was lower than the average. This result was found in 6 (54.55%) out of 11 patients. **Conclusion:** Melan-A expressions on depigmented skins of vitiligo patients are generally below the average value; therefore, adequate intervention is needed to increase the Melan-A expression.

Keywords: vitiligo, Melan-A/MART-1.

Correspondence address: Muhammad Yulianto Listiawan, Department of Dermatology and Venereology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Academic Teaching Hospital, Jl. Prof. Dr. Moestopo No.6-8 Street, Surabaya 60131, Indonesia. Telephone (+6231) 5501609, e-mail: yuliantowawan@yahoo.com.

INTRODUCTION

Vitiligo is a chronic acquired systemic disease, which is characterized by loss of melanocytes in the epidermis, and it involves several genetic and non-genetic environmental factors. Clinical manifestations of vitiligo are milk-white macules, well-defined, may appear with different shapes and sizes in the skin, hair, and mucous membranes.¹⁻³ Currently, the available therapy gives a relatively unsatisfactory result; thus, this disease may promote a decrease in quality of life as it may trigger insecurity, depression, and a high sense of psychosocial discrimination against affected individuals.^{1,2} Vitiligo affects 0.3%-0.5% of the world's population, with increasing prevalence over the last few decades.¹ The data are consistent with a retrospective study conducted by Hutomo S. in 2012, confirming that the prevalence of new vitiligo patients in the Cosmetic Division of Academic General Hospital Dr. Soetomo Surabaya between 2009 and 2011 (3 years) was 0.35% and increased to 0.5% in 2014.⁴

To date, the cause of vitiligo is still unclear. A study proves that cell-mediated immunity plays a role in the pathogenesis of vitiligo. T cells found in inflamed vitiligo suggested that cellular immunity plays a role in the pathogenesis of vitiligo. Some studies hypothesize that vitiligo patients have autoantibodies and autoreactive T cells in

melanocytes.^{6,7,16-17} Melan-A is a melanoma-related antigen that is recognized by autologous cytotoxic T cells and is one of the critical markers for detecting melanocytes. Several studies have shown that Melan-A markers are more specific and sensitive than S-100 and Human Melanoma Black 45 (HMB-45) markers. The S-100 marker, which identifies S-100 protein, is not a specific marker as adipocytes, Schwann cells, and myoepithelial cells also express this marker. The HMB-45 marker, which identifies gp100, is considered more specific than the S-100 marker but does not show absolute specificity because it can also be expressed by sweat gland cells and nonmelanocytic tumors. Based on the specific cellular immune response to melanocytes, recent studies have shown the circulation of Melan-A cytotoxic T lymphocyte cells in the majority of vitiligo patients. T cells express an increase in lymphocyte-related antigen receptors. This correlates with the level of depigmentation and disease activity.^{7-9,13,18} Research by Kubanov *et al* in 2016 regarding Melan-A markers showed that Melan-A was found in the skin of a healthy person and vitiligo patients. However, the expression of Melan-A in vitiligo skin lesions is smaller than healthy skin.⁹ Kholy in 2016 reported that Melan-A is found in the perilesion skin from vitiligo patients, and the expression of Melan-A in vitiligo patients' skin lesions is smaller compared to healthy skin.¹⁰

In this study, we evaluated Melan-A expression, which is colored with Melan-A antigen, found in the skin lesions of vitiligo patients. Vitiligo is a pigmentation disorder based on the loss of melanocytes, decreasing the Melan-A expression in the depigmented skin of vitiligo patients. In the future, we hoped that it could be used as an indicator of vitiligo therapy.

RESEARCH METHODS

This was a descriptive study aimed to evaluate Melan-A expression in the depigmented skin of vitiligo patients. This research was conducted from October 2018 to January 2019 in the Cosmetic Division of Academic General Hospital Dr. Soetomo Surabaya. Each sample was recorded, and biopsy of the depigmented vitiligo lesions was performed. Melan-A immunohistochemical examination was conducted at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. Then the Melan-A expression was calculated in five visual fields, and one of which was one millimeter. The Melan-A was used for the staining itself.

The study samples were the patients of the Cosmetic Division of Academic General Hospital Dr. Soetomo Surabaya. The samples were vitiligo patients who met our inclusion criteria with consecutive sampling. Eleven patients were selected as the subjects of the study. Our inclusion criteria were: vitiligo patients, was not under topical corticosteroids therapy, topical calcineurin inhibitors, topical calcipotriol in the last two weeks, did not consume systemic corticosteroid drugs in the last three months, did not use PUVA, NB-UVB, and excimer laser in the last three months, was not pregnant, more than 21 years old, good general condition, and willing to take part in research. This research has been declared to be ethically feasible by Health Research Ethic Committee of Dr. Soetomo General Hospital on May 5th 2017 with number 329/Panke.KKE/V/2017.

RESULT

The immunohistochemical examination of the depigmented skin showed that there were two patients who did not have Melan-A expression in the five visual fields.

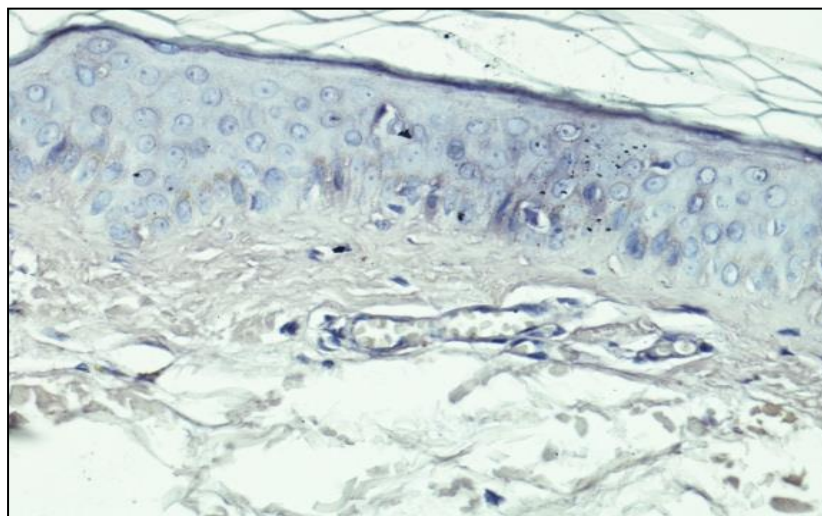


Figure 1. Melan-A expression in depigmented skin of vitiligo patients. The red arrow shows the expression of Melan-A in one field of view; 1000x magnification.

This study found that the average value of Melan-A expression was 1 (1.02) with a minimum value of 0.00, a maximum value of 3.00, and an average value

of 0.8. This study also found that Melan-A expression was below the average, and it was found in 6 (54.55%) patients.

Table 1. Description of Melan-A values

	Mean	Tandard Deviation	Median	Minimum	Maximum
Melan A pre-treatment	1	1,02	0,80	0,00	3,00
				n	%
Melan A category		Below average		6	54,55%
		Above average		5	45,45%

DISCUSSION

Based on research conducted by Kubanov *et al* in 2016 in Russia, the mean value of Melan-A expression on vitiligo depigmentation skin is 0 with a minimum value of 0 and a maximum value of 1; thus, the results are consistent with our findings.⁹ Research conducted by Kholy in 2016 in Egypt also stated that decreased expression of Melan-A was found in the depigmented skin of vitiligo patients compared to healthy skin with $p < 0.01$.¹⁰ This is consistent with our findings as the Melan-A expression in the depigmented skin of vitiligo patients were below average value. Nai *et al* in 2008 in Brazil found that Melan-A marker, in healthy skin, showed melanocytes normally distributed in the basal layer presenting a large number of dendritic processes. In 12 patients, Melan-A showed the absence of melanocytes in depigmented skin. The Melan-A marker showed a statistically significance difference between samples ($p < 0.05$). The study by Carlson *et al* mentioned the Melan-A marker, protein specific to melanocytes and related to melanosome, presents performance higher than HMB-45 for the identification of melanocytes, since HMB-45, an indicator of expression of a protein that shows a presence of melanosomes in stage 1, 2, and active melanogenesis. The antibody Melan-A was more effective to determine the absence of melanocytes because Melan-A showed its absence or decrease in the cases, in addition reduction in cell volume and absence of dendrites, appearing to be even a better marker for the vitiligo diagnosis.¹⁹ The first indications for a possible role of T-Cells in the pathogenesis of vitiligo came from case reports on inflammatory vitiligo. Histopathologic investigations of the perilesional skin suggested the involvement of lymphocytes in the depigmentation process. Immunohistochemical studies have now confirmed the presence of infiltrating T-Cells and their frequent apposition to perilesional melanocytes in this type of vitiligo. Importantly, similar *in situ* T-Cell infiltrates with predominant presence of CD8+ T-Cells have also been detected in common generalized vitiligo. Wijngaard *et al* in 2001 in The Netherlands concluded that recently, Melan-A-specific CD8+ T-Cells marker identified in peripheral blood of vitiligo patients by the tetramer technique. Melan-A is one of the melanocyte-specific differentiation antigens that is recognized by CTL in melanoma. High numbers of Melan-A were observed in 7 of 9 patients with vitiligo.²⁰

Currently, there is no specific index or tools to measure the success rate of vitiligo therapy evaluation and its severity. To obtain consensus data, both evaluation and therapy require a uniform index of vitiligo severity. The method used to diagnose the

severity of vitiligo and evaluating therapy are factors that may affect the quality of life of vitiligo patients. The common method is based on visual observations with diverse interobserver variations, which causes the results to be very subjective.^{11,12,14,15} Immunohistochemical examination of Melan-A can be considered as an indicator of therapeutic success in evaluating vitiligo therapy objectively.

CONCLUSION

This study concluded that Melan-A expressions on depigmented skins of vitiligo patients are generally below the average value; therefore, adequate intervention is needed to increase the Melan-A expression.

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