Section: Dermatology

Original Research Article

IMMUNOHISTOCHEMICAL ANALYSIS OF MELANOCYTE CONTENT IN LESIONAL, PERILESIONAL AND UNINVOLVED SKIN OF VITILIGO USING THE MELAN- A MARKER.

Priyanka Karagaiah[1], Leelavathy B[2], Hitesh S Byatroy[3], Kavyashree K[4]

[1]*Dermatologist, Bangalore Medical College, Bengaluru, India.*
[2]*Professor & Head, Department of Dermatology, Shri Atal Bihari Vajpayee Medical College, Bangalore, India.*
[3]*MBBS MS Orthopedics FRGUHS, Bangalore Medical College, Bengaluru, Karnataka, India.*
[4]*Senior Resident, Department of Dermatology, Akash medical College, Devanahalli, Bangalore, Karnataka, India.*

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Corresponding Author:
Dr. Kavyashree K
Senior Resident, Department of Dermatology, Akash medical College, Devanahalli, Bangalore, Karnataka, India.
Email: kavyakrishnammc13@gmail.com

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ABSTRACT

Background: Vitiligo is an acquired disease characterized by progressive skin depigmentation resulting from an autoimmune response targeting epidermal melanocytes. Few studies have shown specific cytotoxic T-lymphocyte response against Melan – A which is the most specific marker of melanocytes. There are also other studies which have confirmed the presence of residual melanocytes even in the vitiliginous skin. Objectives: To evaluate the melanocyte content in lesional, perilesional [within 2 cm] and distant normal skin of non- segmental vitiligo patients.

Materials and Methods: A total of 40 cases of non-segmental were enrolled in the study based on the prevelance of vitiligo in India. After taking informed consent, skin biopsy from the lesional, perilesional and normal skin was sent to pathology for immunohistochemical analysis of melanocyte content in the above zones using Melan – A marker.

Results: All 40 lesional sites were negative for Melan-A staining. The Melan-A positive melanocytes were reduced in immediate perilesional skin compared to distant normal skin. Paired t-test was applied between perilesional skin and normal skin and the result was statistically significant at $p<0.001$.

Conclusion: Melan-A positive residual melanocytes were not present in the lesional skin in our study as opposed to very few studies which confirmed their presence in the vitiliginous skin even in the long standing cases. But there was significant difference between perilesional and normal skin.

Keywords: Vitiligo, Melan – A, Immunohistochemistry, Cytotoxic T lymphocytes.

INTRODUCTION

Vitiligo is characterized by progressive skin depigmentation resulting from an autoimmune response targeting epidermal melanocytes. As far as the pathogenesis is concerned, some authors claim that epidermal melanocytes are absent in depigmented lesions of vitiligo. Others claim that melanocytes and melanin are present in areas of depigmentation even in long-standing vitiligo lesions. Melan-A–specific cytotoxic T-cells are known to have the phenotype of memory T-cells (CD45RO+) and to express cutaneous lymphocyte antigen (CLA), and so their important role in vitiligo pathogenesis is evident. This study aims to compare the melanocyte content in lesional, perilesional and distant clinically normal skin in patients of vitiligo using the Melan A marker. Our study aims at adding to the existing data, regarding role of involvement of Melan A in the pathogenesis of vitiligo Chronicity may be attributed the presence of memory T cells against MART 1/ Melan A. Further studies has to be done to establish the pathogenesis, with the hope of paving way for development of new molecules or gene therapy targeting MART-1/ Melan A specific cytotoxic T cells.

MATERIAL AND METHODS

Selection of the cases: Based on the prevelance of vitiligo in India ,40 non-segmental vitiligo cases aged above 18 years, who were either not on treatment or on any treatment except phototherapy were enrolled. After taking informed consent, patients were examined clinically and dermoscopically to confirm the diagnosis. Punch biopsy was taken from total three sites in a patient i.e., from lesional skin, perilesional skin (within 2cm of the lesion) and the distant normal skin.

Melan A staining procedure in brief:

Skin biopsy samples were processed at pathology lab. Paraffinised tissue blocks were cut into thin sections of 3-4 microns. The sections were placed in hot air oven over night and deparaffinised with fresh xylene. Later the sections were boiled in pressure cooker with Tris-EDTA buffer. After cooling, the slides were washed with distilled water and 3% hydrogen peroxide was added to inhibit endogenous peroxidise activity followed by washing with TBS buffer. Primary antibody Melan-A (A 103) clone was added and again the slides were washed with TBS buffer. Later secondary antibody was added and a wash with TBS buffer was given. The sections were counterstained with hematoxylin and washed with distilled water in the end.

The slides hence stained were examined under microscope. The total number of Melan-A positive melanocytes per 100 basal keratinocytes were counted in all three sites and results were analysed.

Methodology for stastistical analysis: The results were analysed using Paired t- test.

RESULTS: Among the 40 cases 25(62.5%) were females and 37.5% were males. Out of 40 cases ,5(12.5%) were newly diagnosed and 35(87.5%) were on treatment other than phototherapy. The mean duration of disease was 5.46+1.24 years. Melan –A positive melanocytes were absent from all the 40 lesional sites [Figure 1, 2 and 3]. The mean Melan-A positive melanocytes from perilesional skin was 5.04 cells per 100 basal keratinocytes [Figure 4,5,6]. The mean Melan-A positive melanocytes from distant normal skin was 11.52 cells per 100 basal keratinocytes [Figure 7a and 7b,8,9]. There was a significant difference between perilesional and distant normal skin ($p < 0.001$). But there was no residual melanocytes in the lesional skin indicated by complete absence of Melan A staining in all the 40 patients.

RESULTS

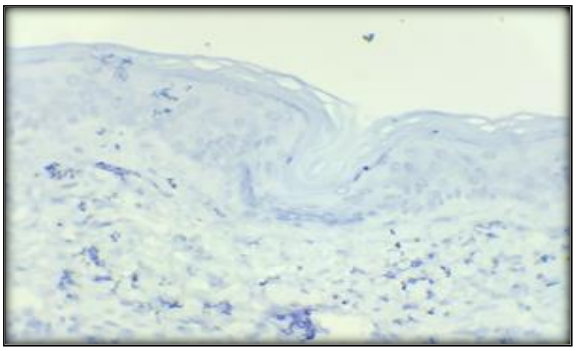

Figure 1: Lesional skin with absence of Melan A melanocytes in the stratum basale (IHC Melan A,200x)

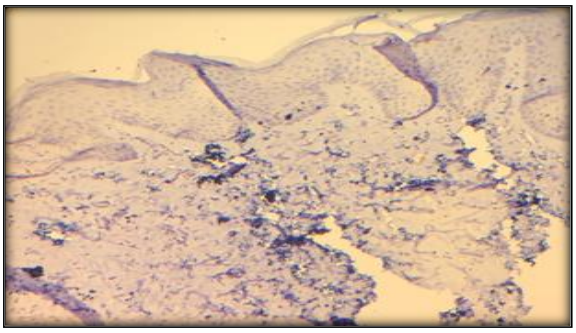

Figure 2: Lesional skin with absence of Melan A melanocytes over the entire basal layer (IHC Melan A,40X)

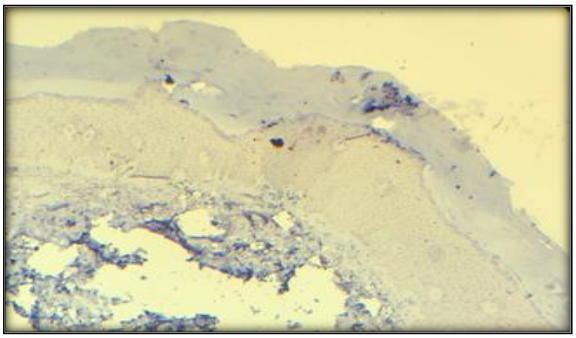

Figure 3: Lesional skin(palms) with total absence of Melan –A melanocytes over the entire basal layer (IHC Melan A,40x)

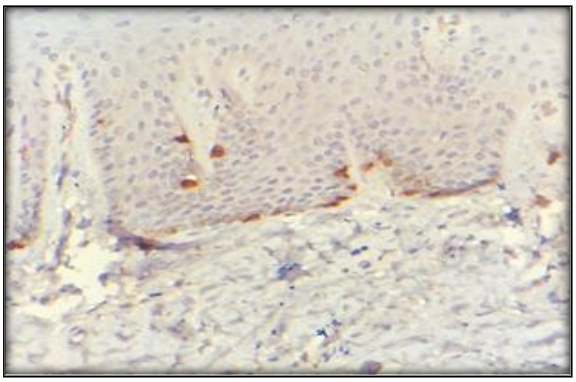

Figure 4: Perilesional skin with few Melan A positive melanocytes at irregular intervals on the basal layer (IHC, 200x)

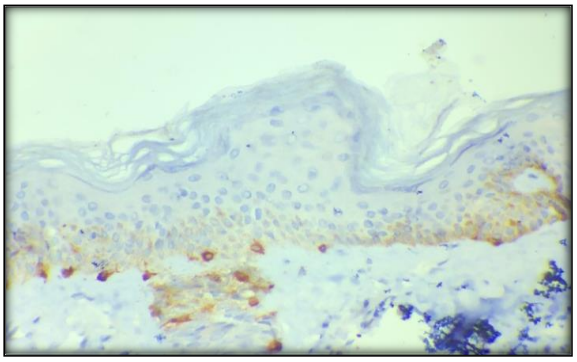

Figure 5: Perilesional skin with Melan A positive melanocytes at regular intervals on the basal layer (IHC,200x)

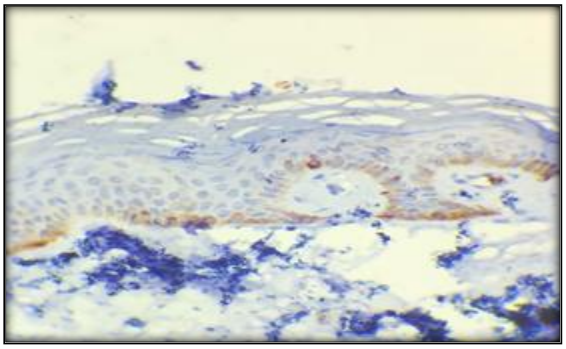

Figure 6: Another perilesional skin with reservoir of Melan A positive melanocytes along stratum basale (IHC,40x)

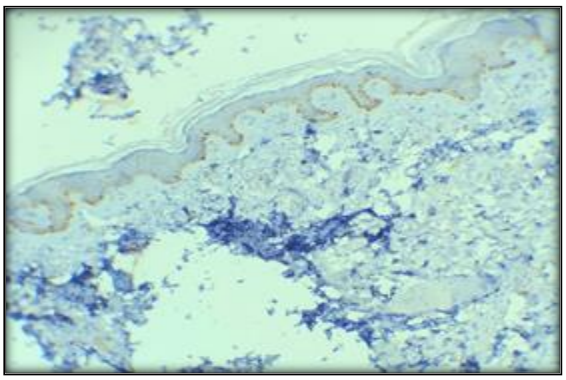

Figure 7a: String of Melan A positive cells along the basal layer of distant normal skin (IHC,10X)

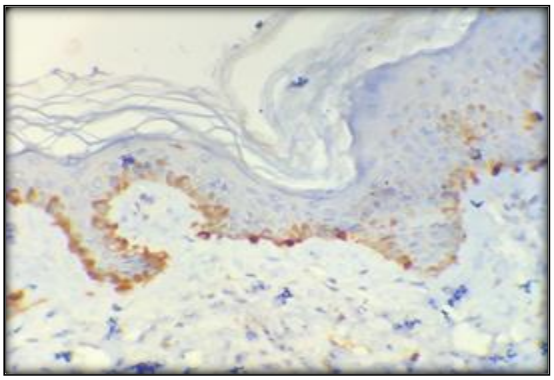

Figure 7b: Numerous Melan A positive melanocytes along the basal layer in the distant normal skin (IHC,40x)

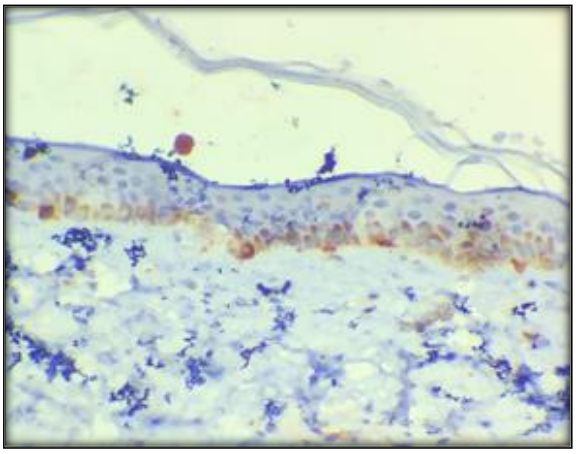

Figure 8: Distant normal skin with multiple Melan A positive melanocytes (IHC,40x)

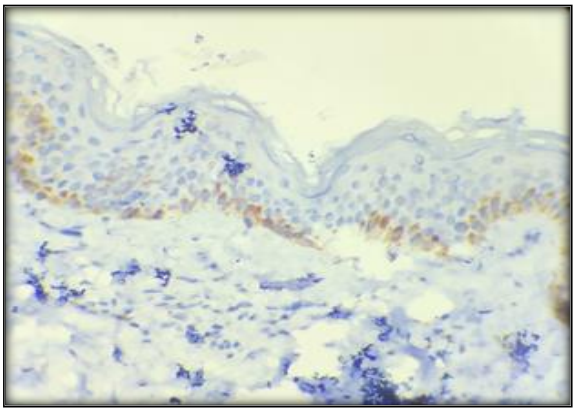

Figure 9: Distant normal skin with many Melan A positive melanocytes along the stratum basale (IHC,200X).

DISCUSSION

Vitiligo is an acquired condition which targets the melanocytes resulting in depigmented patches on the skin. According to Shah et al, prevalence of vitiligo in India is around 0.46% to 8.8%.[1] The pathogenesis is multifactorial and involves autoimmune causes as well as oxidative and mechanical stress. Antimelanocyte antibodies appeared to be elevated in vitiligo patients compared to healthy controls, also implicating immune responses in disease pathogenesis. But histologic studies revealed that CD8+ T cells infiltrated lesional epidermis and were found next to dying melanocytes, strongly supporting T-cell–mediated cytotoxicity as the key event in vitiligo. Cytotoxic T cells have been found in the peripheral blood of vitiligo patients in various studies.[2,3] Bringing all the above hypotheses together 'convergence theory' was proposed.[4] The concept of neural hypothesis has come into existence while studying the characteristics of segmental vitiligo. Currently "Neural hypothesis" remains unsupported by evidence and should be discarded.[5]

Studies using human tissues and a mouse model of vitiligo have revealed that interferon - γ is the key cytokine that drives the disease. IFN- γ is secreted

by melanocyte-reactive autoimmune CD8+ T cells, and induces the production of CXCL10 and other chemokines from keratinocytes, which promote the further recruitment of additional T cells that progressively destroy more melanocytes as the disease spreads.[6] This further potentiates the concept of immune mediated pathogenesis in vitiligo. But the clear cut delineation of which antigen is targeted by T-cells is still obscure.

The basic histopathological finding in vitiligo is the absence of functional melanocytes in the basal layer of the epidermis. But there are again different school of thoughts regarding this finding. Few authors opine that epidermal melanocytes are absent in vitiliginous skin. Tobin et al,[7] confirmed that residual melanocytes were isolated from the depigmented skin of all 16 patients and the vacuolations inside melanocytes was reversible after treating with pseudocatalase and NBUVB. But some authors say melanocytes and melanin are found even in longstanding vitiligo lesions. These confusions may be due to different types of markers selected and the dendritic morphology of melanocytes is not appreciable by light microscopy. There comes the role of immunohistochemistry(IHC) which adds to the sensitivity of detecting melanocytes.[8]

Hence the knowledge about various IHC markers in melanocytes becomes important. MITF (Micropthalmia Associated Transcription Factor) protein is shown to regulate the expression of several immunohistochemical markers, including tyrosinase, Melan-A/Mart1, and HMB-45/gp100/PMEL17.[9] These melanocyte glycoproteins have been shown to be antigenic like Melan- A/MART1, the gp100, tyrosinase etc.

Melan-A, also known as MART-1(Melanoma antigen recognized by T cells-1) is an important melanocyte marker. It plays an essential role in the expression, stability, transport and processing of melanosomes. In a number of studies, Melan-A marker was shown to be more specific and sensitive than the S-100 and HMB-45. Studies have shown that there are specific cytotoxic T lymphocyte responses against Melan- A/MART-1 in lesions of vitiligo. According to Wańkowicz et al , perilesional T-cell clones (TCC) derived from vitilgo patients revealed presence of high frequencies of Melan A–specific CD8 T cells.[10] Poole et al showed that cytotoxic CD8-T cells in the perilesional skin parallels the disappearance of melanocytes.[11] Pelle et al found that these residual melanocytes are much more sensitive to oxidative stress than adjacent keratinocytes.[12] Many studies are undertaken to know the micro environment in the vitiligienous skin. Wagner et al even demonstrated altered adhesion between melanocytes and keratinocytes in the epidermis of vitiligo patients.[13] In the study by Palermo et al in vitiligo patients, the specific cytotoxic T cells against Melan-A/MART1 expressed the skin-homing receptor, cutaneous lymphocyte antigen (CLA).[14]

Recently Alexey Kubanov et al conducted a similar study titled “Immunohistochemical analysis of melanocyte content in different zones of vitiligo lesions using the Melan-A marker”, where they found more than a 3 fold decrease of Melan-A+ melanocytes in perilesional normally pigmented skin of non-segmental vitiligo patients compared with the skin of healthy volunteers. ($p<0.001$). They also confirmed that residual melanocytes were present on all the three zones eventhough the number varied between different zones.[15] Kim et al also showed Melan A+ melanocytes number was significantly decreased in vitiligo skin as compared with normal or nevus depigmentosus skin and they thought Melan- A iimunostains would be helpful to differentiate vitiligo from nevus depigmentosus.[7]

On the contrary, Adams et al found no singnificant role of Melan A specific T cells in the pathogenesis in vitiligo.[16]

But the utility of melanocyte IHC markers is not just limited to vitiligo.They can be used as immunotherapeutical intervention in melanoma. Jager et al found major regression of metastatic melanoma under continued immunization with peptides derived from the melanocyte differentiation antigens Melan A/MART-1. Regression of metastatic melanoma has been observed so far only in response to immunization with a Melan-A/MART-1 peptide.[17] Till date there is no complete cure for vitiligo. Morever the remission is variable with different modalities of treatment. Numerous studies show that patients with vitiligo feel stigmatized, have low self-esteem with poor body image, and suffer a considerable psychosocial burden.[18] In our study,we found that there was complete absence of Melan A + melanocytes in the vitiliginous skin and their number was significantly reduced in the peri lesional skin compared to distant normal skin.We could not find any residual melanocytes in the lesional skin. But more studies are needed to decode the molecular basis of melanocyte destruction and the possible role of targeted therapy against markers like Melan A or repigmentation chances from residual melanocytes can be thoroughly explored as a newer therapeutic modality.

CONCLUSION

Vitiligo is frequently dismissed as “cosmetic,” however
it is often psychologically devastating for patients. Since the depigmentation process can be reversible, either spontaneously or by using various therapeutic approaches, it seems justifiable to readdress the issue and to re-explore a possible reservoir of melanocytes. The detailed knowledge about molecular level pathogenesis can shine some light on developing targeted therapies for vitiligo.

Conflicts of Interest: None
Acknowledgement: None.

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