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Immunohistochemical Markers of Melanocyte Content in Vitiligo Skin

Aya KH.Khamis [1], Essam M.Akl [1], Shymaa M.Rezk [1]and Asmaa G.Abdou[2]
[1] Dermatology, Venereology and Andrology Dept., Faculty of Medicine, Benha University, Egypt
[2] Pathology Dept., Faculty of Medicine, Menoufia University, Egypt
E-mail: ayakhamis991@gmail.com

Abstract
Background: Vitiligo is a common acquired pigmentary skin condition, affecting people all around the world. lesions of vitiligo develop due to loss of functional melanocytes in the affected skin, leading to a decrease in the amount of melanin pigment. Even after depigmentation process has occurred, questions about the existence of remaining melanocytes and the best ways to identify them persist. Objectives: This paper aims to detect if Melan-A , also known as MART-1, is a Melanoma Antigen Recognized by T cells - 1 marker and Human Melanoma Black-45 (HMB-45) marker may be used to diagnose vitiligo by detecting melanocytes in vitiligo skin. Conclusions: Both the detection of melanocytes in vitiligo patients' skin and the estimation of melanocyte concentration may be aided by the use of Melan-A and HMB-45 markers. Since there has not been a single identified marker that stains melanocytes with maximum sensitivity and specificity, immunohistochemical markers are commonly used in combination.

Key words: Vitiligo, Melanocyte, Melan-A marker, HMB-45 marker .

1. Introduction

A persistent pigmentary illness that affects people worldwide is vitiligo. The general incidence rate is between half percent and two percent, and it doesn't discriminate according on age, color, sex, ethnicity, or skin type. Degeneration of viable epidermal melanocytes is the defining feature of vitiligo. [1] There is continuous debate over the many hypotheses concerning the loss of melanocyte function, and the precise pathophysiology remains unknown. Oxidative stress, autoimmune, genetic, and dysfunction of the nervous system are among the many hypothesized pathophysiological mechanisms. Vitiligo is mostly diagnosed by clinical examination; however, there are additional depigmentation illnesses that may be hard to be differentiated from vitiligo that differ from vitiligo in course, prognosis and therapy. Results from the clinical trial to distinguish between these diseases, hematoxylin and eosin (Hx & E) staining and wood's light are not enough. Thus, diagnosis relies heavily on immunohistochemical staining. Immunohistochemical studies of various melanocytic antigen expression in the skin may provide valuable insights into the condition of melanocytes in vitiligo lesions. So, vitiligo melanocyte identification has been proposed using a variety of markers. S-100, Melan-A, and HMB-45 are the most important immunohistochemical markers that are often used to diagnose melanocytic lesions, but none of them are really sensitive or particular. [2]

Vitiligo

Vitiligo is a common pigmentary dermatological condition that manifests clinically as well-demarcated, non-scaly milky white macules and patches. It results from selective degeneration of melanocytes from the epidermis. It occurs in different parts of the body on the skin and sometimes also on the mucous membranes. [3]

Epidemiology

with an approximated frequency of half –two percent of the global population worldwide. Vitiligo is the most common depigmenting skin condition. In Egypt, it affects 0.06% of people aged between six – twelve years. Both sexes are equally affected, but the societal stigmatization of women and girls may explain why they seek help more often than boys and men. Vitiligo manifests in twenty five percent of cases before the age of ten, in nearly fifty percent of cases before the age of twenty, and in around seventy-eighty percent of cases before the age of thirty. [4]

What causes vitiligo?

The loss of healthy epidermal melanocytes is one of several symptoms of vitiligo, a complex condition. Several elements interact in a complicated way to determine the precise pathophysiology. There are a number of hypothesized pathophysiological processes, including oxidative stress, genetics, autoimmunity, and dysfunction of the neurological system. [5]

1. Genetic hypothesis

Vitiligo is associated with clustering in families. Multiple studies have shown that vitiligo frequency is 0.14 percent to 20 percent among first-degree relatives. However, only 23 percent of monozygotic twins demonstrated concordance, suggesting that vitiligo's etiology may be influenced by factors other than genes. Vitiligo is a polygenic disorder with

multiple identified genes. including the major histocompatibility complex (MHC), angiotensin-converting enzyme (ACE), catalase (CAT), cytotoxic T lymphocyte antigen-4 (CTLA-4), interleukin-2 receptor A (IL2RA), Protein Tyrosine Phosphatase Non-Receptor Type 22 (PTPN22), X-box binding protein 1 (XBP1) and fork-head box P1(FOXP1). [6]

2. autoimmune hypothesis

Autoimmune hypothesis is the most popular and well-established hypothesis which suggests that a disruption in the immune response causes melanocytes to be degenerated by autoimmune mechanisms, either autoantibodies targeted to melanocyte surface antigens or cytotoxic T cells. For instance, vitiligo usually occurs with other autoimmune disorders; for example, vitiligo is usually associated with thyroid disorders including Hashimoto's thyroiditis and Graves' disease, as well as Addison's disease, alopecia areata and systemic lupus erythematosus. [7]

1. Oxidative stress hypothesis

This hypothesis was supported by the fact that melanocytes are damaged by certain intermediates (like dopaquinones and indoles) during melanin production. In vitiligo patients, systemic oxidative damage occurs due to imbalance between their enzymatic and non-enzymatic antioxidant systems. Oxidative stress may occur in segmental vitiligo due to low catalase levels; in non-segmental vitiligo, it may be due to low glutathione peroxidase levels and decreased glutathione. [8]

2. Neurochemical hypothesis

The communication between the skin and nervous system has been suggested by a number of clinical findings Firstly, vitiligo patches in the segmental type of vitiligo are almost dermatomal in distribution and the nonsegmental vitiligo they are symmetrical vitiligo. Furthermore, Patients with diabetic neuropathy and transverse myelitis are also known to develop vitiligo. Finally, vitiligo may be triggered or worsened by intense mental stress. (12) melanocytes are destroyed by Acetylcholine (Ach) and norepinephrine (NE), By disrupting cellular sulfhydryl groups, limiting mitochondrial calcium absorption, and suppressing melanogenesis, NE directly causes Melano cytotoxicity. [9]

1. Adhesion defect hypothesis (Melanocytorrhagy hypothesis)

One possible explanation for the loss of melanocytes in vitiligo lesions is defects in adhesions of melanocytes. The theory suggests that vitiligo forms at trauma sites because weakly anchored melanocytes, when exposed to even little friction or stress, separate from the basement membrane, migrate upward across the epidermis, and then disappear into the environment. This helps to explain the Koebner's phenomenon. [10]

2. The biochemical hypothesis

It is believed that dysregulation of biopterin pathways predisposes to vitiligo and melanocyte toxicity. Vitiligo is associated with higher levels of teridines (6R)-L-erythro 5, 6, 7, and 8 tetrahydrobiopterin (6BH4) and (7R)-L-erythro 5, 6, 7, and 8 tertahydropterin (7BH4). (11) The enzyme that changes dietary phenylalanine into tyrosine, phenylalanine hydroxylase, requires tetrahydrobiopterin as a cofactor. The metabolic pathway is driven forward by increased 6BH4, which results from either overactivity of its synthesizing enzyme GTP cyclohydrolase I or decreased activity of its recycling enzyme 4a-hydroxyBH4 dehydratase. This leads to a buildup of byproducts 7BH4 and H2O2. Elevated 7BH4 further contributes to an increase in 6BH4, which is lethal at high quantities, by inhibiting phenylalanine hydroxylase **(Figure 1)**. [12]

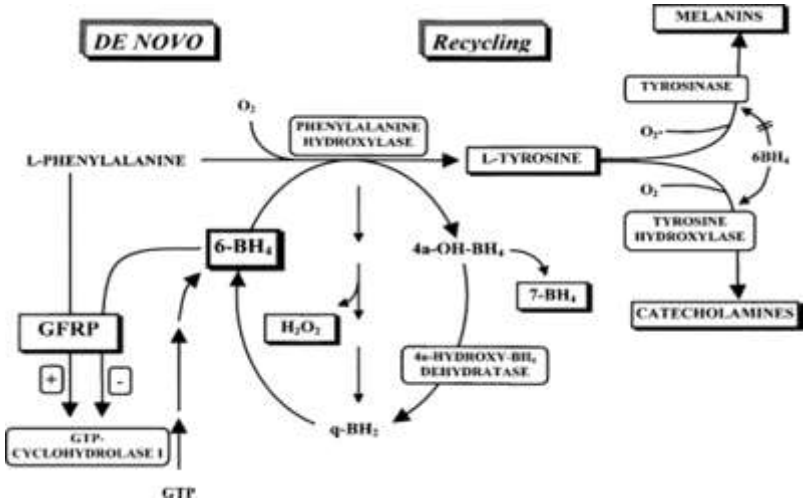

Fig. 1: The scheme represents the biopterin metabolic pathway and network, defective synthesis of 6BH4 leading to increased 7BH4 production in vitiligo. [13]

1. Apoptosis and accelerated cell senescence hypothesis

Dysregulation of apoptotic regulatory molecules is linked to vitiligo. B-cell lymphoma protein-2 (Bcl-2) can protect cells from numbers of apoptotic stimuli, while Bcl-2 Associated X (Bax) is an apoptosis agonist. A cell's sensitivity to apoptosis induction is determined in part by its Bcl-2/Bax ratio, as apoptosis is initiated when pro-death signals surpass pro-survival signals. According to the findings, perilesional melanocytes had a lower Bcl-2/Bax ratio than the control melanocytes. Patients with vitiligo may have melanocytes that are more amenable to apoptotic activation due to changes in Bcl-2 and Bax expression. [14]

2. Viral hypothesis

Autoimmune hepatitis and chronic hepatitis C virus infection are linked to vitiligo. Vitiligo patients had low levels of hepatitis B virus seropositivity. The severity of vitiligo or its etiopathogenesis may be exacerbated by previous or current Cytomegalovirus infections. On top of that, the link between vitiligo and other viruses including herpes, hepatitis E, Epstein-Barr, and human immunodeficiency virus is still obscure. [15]

3. The convergence hypothesis

The hypothesis of convergence states that all the above theories; genetic, neurochemical, apoptotic, autoimmunity, melanocytorrhagy, altered cellular environment and impaired melanocyte migration, are all components of the pathophysiology of vitiligo and none are exclusive. [16]

Classification of vitiligo

1) Clinical classification [17]

I. Localized

- **Focal:** One or more macules located in a specific spot describe focal dermatoses.
- **Segmental:** One or more macules arranged in a dermatomal or quasi-dermatomal pattern characterize the segmental form. SV usually stops suddenly near the midline and is unilateral. Kids are the ones who get it the most often.
- **Mucosal:** just the mucous membranes are impacted.

II. Generalized

- **Acrofacial:** Distal finger and toe tips, as well as periorificial regions, could have acrofacial depigmentation
- **Vulgaris:** Dispersed patches that are widely spaced
- **Mixed:** Mixed vitiligo includes both acrofacial and vulgaris forms, as well as segmental and acrofacial forms.

III. Universal

Complete or nearly complete depigmentation, that is often associated with multiple endocrinopathy syndromes.

2) Classification according to progress, prognosis, and treatment [18]

There are two main clinical kinds of vitiligo: segmental and non-segmental**.**

- **Segmental:** typically starts early and spreads quickly throughout the impacted area. Segmental vitiligo may halt in its tracks, and depigmented areas may remain unaltered for an extended period.
- **Non-segmental:** comprises all forms of vitiligo, with the exception of segmental vitiligo.

Immunohistochemical markers

The S-100 protein is the most commonly used marker for melanocytic lesion diagnosis, it has low specificity (between 75 and 87 percent) and excellent

sensitivity (between 97 and 100 percent). This is due to the fact that it serves as a marker for normally functioning lymphocytes, skeletal and cardiac striated muscle, Schwann cells, normal melanocytes, histiocytes, and chondrocytes. Therefore, other markers, such as HMB-45 and Melan-A are required for more precise diagnosis. [19]

Melan-A Marker

Chemistry: One of the most significant melanocytic indicators is Melan-A marker. The MLANA gene, located on chromosome 9p24-1, encodes a protein of 118 amino acids and a single domain that spans about eighteen kDa. [20] According to messenger RNA analysis in healthy human cells and tissues, Melan-A is an antigen exclusive to the melanocytic lineage that is expressed only by skin and retinal melanocytes. Reports indicate that it can also stain melanophages, revealing cytoplasmic staining in the absence of background staining, in addition to melanocytes. The premelanosome protein 17 (PMEL17), which is essential for the development of stage two melanosomes, is mostly found in melanosomes and the endoplasmic reticulum, and it is involved in many aspects of this process, including expression, stability, transport, and processing. [20]

Source: a number of monoclonal antibodies from mice that are effective against recombinant Melan-N immunogen and can be applied to tissue samples that have been formalin-fixed and regularly treated. An antibody clone against the Melan-A protein (M2-7C10) and an antibody clone against the Melan-A recombinant protein (A103) are two antibodies that are available for purchase. The available data suggests that both antibodies are equally sensitive to various melanocytic lesion types. [21]

Physiological role in melanocytic diseases:

Due to its ability to specifically stain cells of the melanocyte line and to exclude other cells from the background, Melan-A is a useful diagnostic indicator of lesions of melanocytes. It aids in proper identification of melanocytic lesions by confirming their origin and differentiating them from their near mimickers. [22] Melan-A expression has been demonstrated by immunohistochemistry studies in lymph node capsular nevi, as well as in all types of nevi, including congenital, compound, junctional, intradermal, dysplastic, and Spitz nevi. the majority of primary and metastatic melanomas also express it. the primary melanomas have shown Melan-A expression percentages of 85% to 97% and metastatic melanomas of 57% to 92%. In sentinel lymph nodes, Melan-A is thought to be a helpful marker for identifying micrometastasis due of its increased melanoma sensitivity. It is easier to interpret the immunostaining because, in contrast to S100 protein, it is not expressed by lymph node dendritic cells. [23]

Because autoreactive circulating cytotoxic T-lymphocytes were detected in patients' blood for this melanosomal protein, Melan-A, it is of great interest to study its expression in vitiligo lesions. Because of their Melan-A-specific cytotoxic T-lymphocyte nature and expression of cutaneous lymphocyte antigen (CLA), making their significance in the pathophysiology of vitiligo obvious. [24] the Immunohistochemical studies, which have used Melan-A marker to demonstrate melanocytes in vitiligo lesions and normal skin of healthy volunteers, Reported that the Melan-A marker is present in the skin of healthy volunteers, but in the skin of vitiligo patients, the amount of Melan-A+ cells is decreased. [25] [26]

HMB-45 MARKER

Chemistry: The name "human melanoma black" (HMB) is derived from the immunogen's description. The type 1 membrane-bound melanosomal protein product of the SILV gene on chromosome 12q13-q14 is known as PMEL17. HMB-45 is able to identify this protein. Melanosome structure is largely dependent on the PMEL17 protein. [27] The fibrillar matrix in stage two melanocytes is the primary target of HMB-45 reactions, according to immunoelectron imaging. Multivesicular stage one melanosomes are a secondary target. Stage three melanosomes deposit melanins into these fibrils, which show as intraluminal striations in stage two melanosomes under electron microscopy. This causes the melanosomes to thicken and blacken. [28]

Source: The monoclonal cytoplasmic antibody HMB-45 was first created using an extract from malignant melanoma. The melanosomal protein, which is bound by this antibody, is often produced by cells that is immature or undergoing cell proliferation. It is therefore positive for both fetal and activated melanocytes, confirming melanocyte activity and serving as a highly selective marker for melanocyte differentiation. [29]

Physiological role in melanocytic diseases:

The HMB-45 does not attach to normal melanocytes in adults, but it does bind to cutaneous fetal melanocytes and retinal pigmented epithelium in fetuses and infants. Additionally, it reacts with compound nevi's junctional component and junctional nevi, but not with intradermal nevi, compound nevi's deep component, or nevi involving lymph nodes. Approximately 80% to 10% of dysplastic nevi, 85% to 100% of Spitz nevi, 85% to 100% of blue nevi, and 60% to 85% of congenital nevi test positive for HMB-45. It should be noted that the majority of nevi only show HMB-45 labeling in the top dermal layer (papillary dermis) or adventitial dermal layer surrounding the skin adnexa; cells farther down the dermal layer do not show any

staining. This differs from primary cutaneous melanomas, which often have a patchy staining pattern that labels cells both superficially and deeply inside the lesion. Thirdly, HMB-45 marker expression varies; it ranges from 77% to 100% in original melanoma and 58% to 83% in metastasis. Tumors having epithelioid shape are the most readily stained. The majority of spindle cell melanomas, especially the desmoplastic kind, come back negative. [30]

A previous study in vitiligo patients effectively treated with fractional CO- laser combined with 5-fluorouracil showed that HMB-45 expression revealed few or absent melanosomes, while after treatment, HMB-45-positive cells were detected in the basal cell layer of the epidermis. [31]

HMB-45 was determined to be more specific than Melan-A in benign melanocytic lesions, with higher staining at the dermo-epidermal interface, although it lacked sensitivity. Melan-A was also more sensitive and shown widespread melanocyte staining in benign melanocytic tumors. Keratinocytes and melanophages that were substantially melanized may also be stained with Melan-A. [32] When used in situ, HMB-45 may distinguish between senile lentigo and early melanoma, even though it did not stain as many melanocytes as Melan-A. Because HMB-45 reacts with cytologic atypia in lesions, it has been effectively used to distinguish pigmented actinic keratosis from lentigo maligna. [33]

3. **Conclusions**:

Immunohistochemical markers are commonly used to identify melanocytes in vitiligo patients' skin and to estimate their content in vitiligo lesions. the HMB-45 stain and the Melan-A stain are helpful diagnostic tests for vitiligo that is difficult to distinguish clinically and histologically.

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