Evaluation of High Mobility Group Box 1 In Vitiligo Patients

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Abstract

Background: Vitiligo is an idiopathic acquired disorder of pigmentation of skin and occasionally mucous membranes that is characterized by well defined, depigmented/achromic macules and patches. Vitiligo occurs secondary to a selective and progressive process of melanocytes destruction. Vitiligo is attributable to loss of functional melanocytes and is the most common acquired depigmenting disorder. The nonhistone high mobility group box 1 (HMGB1) DNA binding protein is a physiological activator of immune responses, cellular proliferation and cell death. Although it is implicated in the pathogenesis of autoimmune diseases and cutaneous disorders, the precise role of HMGB1 in melanocytes has yet to be studied.

Keywords: High Mobility Group Box 1, Vitiligo.

Background

Vitiligo is an idiopathic acquired disorder of pigmentation of skin and occasionally mucous membranes that is characterized by well defined, depigmented/achromic macules and patches. Vitiligo occurs secondary to a selective and progressive process of melanocytes destruction. The disease is typically asymptomatic. However, the resulting pigmenitary disfigurement can be quite traumatic, especially when it involves the face, hands, and genitals particularly among patients from dark skinned ethnic groups (1).

Epidemiology

Approximately 0.5% to 1% of the population is affected, and almost half present before 20 years of age. Its prevalence appears to be equal between men and women, and there is no difference in rates of occurrence according to skin type or race. India is considered to have the highest prevalence in the world, at about 8.8%. Men and women are equally affected, but women are more likely to seek treatment (2). The mean age of onset is earlier in those with a positive family history, which ranges from 2% to 7.7%. Vitiligo is significantly more prevalent in young women (30 years of age) than young men. The peak in females occurs in the first decade of life while male peak prevalence is in the fifth decade of life (3).

Diagnosis

Vitiligo usually begins insidiously with an indolent course; lesions wax and wane but tend to coalesce sometimes eventually involving the total body surface area. Severe sunburn, pregnancy, skin trauma, and/or emotional stress may precede onset. Vitiligo is generally slowly progressive, either by centrifugal expansion of current lesions and/or the appearance of new lesions. Classically, discrete uniformly white macules or patches are surrounded by normal skin. Though typically asymptomatic, itch has been reported to precede lesions. Vitiligo frequently occurs at sites that are normally hyperpigmented, including the face (periorificial), the dorsal surface of the hands, nipples, axillae, umbilicus, sacrum and inguinal/anogenital regions. Lesions typically develop in areas of friction, reflecting koebnerization (4).
Diagnosis of vitiligo is usually made clinically and with the use of a Wood’s lamp (a handheld ultraviolet (UV) irradiation device emitting ultraviolet A (UVA) waves at a wavelength of approximately 365 nm) which may also facilitate monitoring the progress of lesions over time (5).

The British Association of Dermatologists proposed recommendations for the diagnosis and evaluation of vitiligo patients (Table 1) (6).

Table 1: British Association of Dermatologists Recommendations for the diagnosis and evaluation of vitiligo patients, (6).

<table>
<thead>
<tr>
<th>• Vitiligo diagnosis is straightforward when presentation is classical.</th>
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<tr>
<td>• When presentation is atypical, cases should be referred for expert assessment by a dermatologist.</td>
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<td>• In adults with vitiligo, a blood test to check thyroid function should be considered</td>
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<td>• A Wood’s lamp may be of use in determining extent and activity of vitiligo, as well as monitoring response to therapy.</td>
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<td>• Response to treatment in vitiligo should be considered in context of the natural history, recognizing that spontaneous repigmentation may occur but is uncommon.</td>
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<td>• Clinicians should assess the psychological and quality of life effects of vitiligo on patients.</td>
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<td>• In clinical trials of vitiligo, the patient’s improvement in (QOL) should be the most important outcome measure.</td>
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Histopathology
Histopathologic evaluation may help differentiate vitiligo from other disorders in ambiguous cases. Melanocytes are absent, and there is a scanty inflammatory cell infiltrate. Melanocytes on the pigmented edge of vitiliginous skin are larger, often vacuolated, and with long dendritic processes filled with melanin granules (7).

Immunohistochemical staining verifies the complete absence of melanocytes in skin that may still have melanin granules within keratinocytes; useful special stains include DOPA, which detects active melanocytes, Mel-5 (anti-TRP1, Anti tyrosine related protein antibody), and NKI/beteb (anti-pMel-17, Anti-Melanoma Associated Antigen 100+ / 7 kDa antibody), which detect both active and dormant melanocytes (8).

In addition, black hairs within vitiligo patches may contain melanocytes. Disease duration is inversely correlated with the melanocytes’ presence within hair follicles. Melanocytes are absent from 100% of white hairs (9).

Clinical variants
Trichrome vitiligo has a tan zone of varying width between normal and depigmented skin. On histopathology, this intermediate tan zone has more inflammatory cells, Langerhans cells, and melanophages than vitiliginous or normal skin; the number of melanocytes is greater than in vitiliginous skin but fewer than in normal skin. (10).
Quadrichrome vitiligo has additional marginal or perifollicular hyperpigmentation; it is more common in darker skin types and in areas of repigmentation. (Blue vitiligo has a blue-grey hue because of the absence of epidermal melanocytes and the presence of numerous dermal melanophages(11). Inflammatory vitiligo or vitiligo with raised inflammatory borders describes erythema at the margins of depigmented macules. A full body skin examination is necessary to detect genital depigmentation if present (4).

**Classification**

According to distribution of the lesions two main types of vitiligo are recognized: generalized and localized. The generalized type presents mostly with macules distributed on either sides of the body, which may be symmetrical or asymmetrical in distribution. If it is of moderate extent, it is termed vitiligo vulgaris, and if it is very extensive it is termed universal vitiligo. It may involve the face and distal extremities, then the term acrofacialvitiligo is applied (8).

Localized type is less common than the generalized type and is further divided into focal, where one or several macules are in one anatomical area, or segmental vitiligo, where one or several macules are in dermatomal distribution (10).

<table>
<thead>
<tr>
<th>Generalized</th>
<th>Localized</th>
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<tbody>
<tr>
<td>Acrofacial</td>
<td>Segmental</td>
</tr>
<tr>
<td>Vulgaris</td>
<td>One or more macules in dermatomal distribution</td>
</tr>
<tr>
<td>Universal</td>
<td>Focal</td>
</tr>
<tr>
<td>Mixed</td>
<td>One or more isolated macules in one general anatomic area</td>
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Table (2): Types of vitiligo,(10)

The Vitiligo Global Issues Consensus Conference (VGICC) used the classification proposed by Taieb and Picardo (2) as a working template. Vitiligo has been classified based on clinical grounds into two major forms, namely, segmental vitiligo (SV) and non-segmental vitiligo (NSV). The term ‘non-segmental vitiligo’ is currently used as a term for different clinical subtypes of vitiligo that are all clearly distinct from SV including acrofacial, generalized, mucosal (multifocal), and universal vitiligo(12). Mucosal vitiligo typically refers to the involvement of the oral and / or genital mucosae. When presenting in isolation, especially for genital involvement, a differential diagnosis of lichen sclerosus should be addressed by biopsy (12).

Other unclassified or poorly classified generalized vitiligoid conditions include punctate vitiligo and other distinct conditions that may be difficult to distinguish from vitiligo clinically include idiopathic guttate hypomelanosis and progressive macular hypomelanosis (12). Punctate vitiligo’ (13) refers to pea-sized depigmented macules that may involve any area of the body.
Table (3): classification of vitiligo

<table>
<thead>
<tr>
<th>Type of vitiligo</th>
<th>Subtypes</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Non-Segmental Vitiligo (NSV)</td>
<td>Focal, mucosal, acrofacial, generalized Universal</td>
<td>Subtyping may not reflect a distinct nature, but useful information for epidemiological studies.</td>
</tr>
<tr>
<td>-Segmental Vitiligo (SV)</td>
<td>Focal, mucosal, unisegmental, bi- or multisegmental</td>
<td>Further classification according to distribution pattern possible, but not yet standardized.</td>
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<tr>
<td>-Mixed Vitiligo</td>
<td>According to severity of SV</td>
<td>Usually the SV part of mixed vitiligo is more severe.</td>
</tr>
<tr>
<td>-Unclassified</td>
<td>Focal at onset, multifocal asymmetrical non-segmental, mucosal (one site)</td>
<td>This category is meant to allow, after a sufficient observation time (and if necessary investigation), to make definitive classification.</td>
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Clinically, NSV is characterized by depigmented macules that vary in size from a few to several centimeters in diameter, often involving both sides of the body with tendency toward symmetrical distribution. Involvement of the scalp and other hair-bearing areas may manifest with localized patches of gray or white hairs. Contrary to SV, in NSV, body hairs are usually spared and remain pigmented, although hair depigmentation may occur with disease progression (14). Segmental vitiligo typically has earlier age of onset than non-segmental vitiligo. SV typically has a rapidly progressive but limited course, depigmentation spreads within the segment over a period of 6–24 months and then stop; further extension is rare. In addition, in contrast to NSV, SV has early involvement of melanocytes of hair follicles, with up to 50% of SV patients exhibiting poliosis in affected areas. In addition, melanocyte autografts typically yield good results in SV patients, with stable repigmentation (15).
Table (4): Segmental versus non-segmental vitiligo (34).

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<th></th>
<th>non-segmental</th>
<th>Segmental</th>
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<tr>
<td><strong>Prevalence</strong></td>
<td>72-95%</td>
<td>5-28%</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>Symmetrical, non-dermatomal</td>
<td>Unilateral, dermatomal</td>
</tr>
<tr>
<td><strong>Onset</strong></td>
<td>Any age</td>
<td>Early</td>
</tr>
<tr>
<td><strong>Course</strong></td>
<td>Variable rate of growth with new lesions throughout life</td>
<td>Initial growth with non-progression within 2 years</td>
</tr>
<tr>
<td><strong>Etiology</strong></td>
<td>Autoimmune</td>
<td>Neurochemical</td>
</tr>
<tr>
<td><strong>Koebnerization</strong></td>
<td>Frequent</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>Autoimmune association</strong></td>
<td>Strong</td>
<td>Rare</td>
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A subtype of vitiligo, termed occupational or contact vitiligo is increased in individuals who are exposed to materials containing phenolic derivatives, such as 4-tertiary butylphenol (4-TBP). Such substances exhibit their action most probably through competitive inhibition of tyrosinase enzyme (16).

**Pathogenesis**

Although vitiligo is extensively studied in the past decades, its exact etiology is still unknown. Many causal factors are involved in vitiligo: deficiency in unidentified melanocyte growth, an intrinsic defect of melanocytes adhesion or genetic factors (17). However, three main prevailing theories of pathogenesis are centered on neurochemical, autoimmune and oxidative stress aspects (30). The convergence theory states that stress, accumulation of toxic compounds, infection, autoimmunity, mutations, altered cellular environment, and impaired melanocyte migration can all contribute to pathogenesis. Autoimmune mechanisms often underlie generalized vitiligo, while a more localized phenomenon may be responsible for segmental or focal vitiligo (i.e. the neurohumoral hypothesis) (31).

1) **Oxidative stress hypothesis:**

Oxidative stress is considered to be the initial pathogenic event in melanocyte destruction. It is unclear till now why both lesional and nonlesional skin from vitiligo patients have abnormally low levels of catalase enzyme, which correlates with high H$_2$O$_2$ levels observed in the epidermis of active vitiligo patients (18). A single nucleotide polymorphism in the catalase gene may interfere with the assembly and function of the enzyme. The deranged melanin synthesis pathways involving 6-biopterin produce high levels of H$_2$O$_2$. Other possible explanations include increases in norepinephrine (NE) and monoamine oxidase, H$_2$O$_2$ generation as a byproduct, and reduced glutathione peroxidase activity (19).

2) **Neurochemical hypothesis:**

Immunohistochemical stains show an increase in neuropeptide Y intralesionally and perilesionally. Vitiligo lesions may also exhibit increased levels of norepinephrine, and a decrease in parasympathetic acetylcholine esterase activity. The increased neurotransmitters may be directly cytotoxic to the cells, or
may have an indirect effect through local vasoconstriction leading to hypoxia and subsequent stress-generated hydrogen peroxide (H2O2) (20).

The source of excess neurotransmitters is uncertain, because both terminal nerve endings and keratinocytes are capable of synthesizing and secreting them. A high local concentration of norepinephrine (NE) has been related to a decrease in phenylethanolamine-N-methyltransferase activity and increased activity in tyrosine hydroxylase (29).

Dysregulation of the nervous system, either at a local or systemic level, may damage melanocytes in vitiligo. In support of this, both melanocytes and nerves arise from neural crest cells, and some vitiligo is segmental, follows the distribution of nerves, and shows alterations in perspiration and changes in nerve structure (24).

There is evidence that this neural dysregulation is systemic and that vitiligo often emerges during periods of increased stress. Changes in serum levels of epinephrine and NE are present, but their significance is unclear (21).

High levels of catecholamines explain the increased intracellular enzymatic activity of catechol-o-methyltransferase, which normally neutralizes the neurotransmitters and generates toxic byproducts. These byproducts can then damage the cell (22).

3) Autoimmune hypothesis:

The first clue to vitiligo pathogenesis came from the description and illustration of a patient with concomitant vitiligo, adrenal insufficiency and pernicious anemia indicating a link between these three autoimmune diseases. The presence of antimelanocyte and antikeratinocyte antibodies in the sera of vitiligo patients also supports the hypothesis. Moreover, there is substantial evidence for the immune mediated destruction of melanocytes; melanoma patients who develop hypopigmentation have a better prognosis, indicating that a common immune response to melanocytes is responsible for both hypopigmentation and tumor control (25, 26).

The humoral immune response in vitiligo is evidenced by the presence of circulating antibodies in the sera of vitiligo patients that are categorized as antibodies to cell surface pigment cell antigen, intracellular pigment cell antigen and non-pigment cell antigen (common tissue antigen) (26). The selective destruction of melanocytes might be due to antibody reactivity directed to the antigens preferentially expressed on pigment cells or from antibody response against antigens expressed on a variety of cell types that might selectively destroy melanocytes because they are intrinsically more sensitive to immune mediated injury than other cells (27).

Autoantibodies against pigment cells might result from a genetic predisposition to immune dysregulation at T-cell level. Alternatively, cross-reacting antigens expressed either on other target cells or infecting microorganisms could elicit autoantibody production. Vitiligo antibodies could also result from an immune response to melanocyte antigens released following damage to pigment cells by other mechanisms and these antibodies might then exacerbate the condition (27).

Lastly, antibodies to SOX9 and SOX10 (transcription factors involved in the differentiation of cells derived from the neural crest) were detected in vitiligo patients without concomitant disease, which suggests a potential general role in vitiligo (32).

Vitiligo patient sera are able to damage melanocytes in vitro both by complement activation and by antibody-dependent cellular cytotoxicity. A positive correlation was observed between the level of melanocyte antibodies and the disease activity. Circulating organ specific autoantibodies particularly to thyroid, adrenal and gastric glands are commonly detected in the sera of vitiligo patients (33).
The cellular immune response in vitiligo is evidenced by histopathological investigation of the perilesional skin of vitiligo suggesting an involvement of lymphocytes in the depigmentation process. Immunohistochemical studies have confirmed the presence of infiltrating T-cells with an increased CD8/CD4 ratio (22). A substantial number of infiltrating T-cells express the cutaneous lymphocyte antigen-CLA, typical of skin homing T cells and a recent study has shown CLA positive cytotoxic-T cells in perilesional skin. These T cells also express activation molecules interleukin-2 (CD25), major histocompatibility complex II (specifically HLA-DR), and secrete interferon-gamma (IFN-γ), which promotes T cell migration to the skin by increasing intracellular adhesion molecule-1 expression (23).

The peripheral blood of vitiligo patients shows high frequencies of Melan-A specific CD8+ T cells with cutaneous lymphocyte antigen, and their number may correlate with disease extent. Moreover, immunization with Melan-A peptide to augment the immune response to melanoma induced vitiliginous areas and regression of the melanoma associated with an oligoclonal expansion of Melan-A/MART-1 specific CD8+ T cells in both the serum and lesions in one patient (33). The above findings suggest a direct evidence for the T-cell mediated melanocyte destruction in vitiligo patients.

Fig.(1): Summary of the cellular and humoral immune mechanisms in vitiligo. Melanocytes damage can be caused by T-cell mediated cytotoxicity or humoral immune response. Intrinsic and extrinsic melanocyte damage leads to lysis of melanocytes. The released content can be presented by MHCII pathway leading to the activation of T-helper cells which further activate CD8+ cells resulting in cell cytotoxicity. Autoantibodies against melanocyte proteins result in antigen-antibody complex formation leading to complement activation or ADCC (Antibody Dependent Cell cytotoxicity) (23).
**Role of HMGB1 in vitiligo:**

**Kim et al.** (28) outlined a possible association between high-mobility group box 1 (HMGB1) and vitiligo. They suggested the potential role of HMGB1 from keratinocytes in melanogenesis and demonstrate that secretion of HMGB1 from neighbouring keratinocytes influences melanocyte survival and the expression of melanogenesis-related molecules. It has been shown that keratinocytes and their products are necessary for the function of melanocytes in the epidermis4 and play a prominent role in the melanocytic death process. (28).

External stressors, such as ultraviolet B radiation and oxidative stress, both regarded as precipitating factors in vitiligo, stimulate HMGB1 release from keratinocytes leading to melanocyte apoptosis and decreasing expression levels of melanogenesis-related molecules. Moreover, electron microscopy analysis shows, in HMGB1-treated cells, the presence of melanosomes trapped in autophagosomes, demonstrating that HMGB1 suppresses melanogenesis via decreasing expression of melanogenesis-associated molecules, including the melanosomal gp100 protein, and melanin, engulfed by autophagosomes. These data were also confirmed by small-interfering RNA gene silencing (28).

By ex vivo skin organ culture experiments, **Kim et al.** (28) demonstrated that HMGB1-treated skin shows enhanced expression of cleaved caspase-3 apoptotic marker compared with control skin. These findings have been also confirmed in lesional and nonlesional skin from patients with vitiligo. Moreover, during melanocytic apoptosis, autophagy is activated to protect melanocytes from HMGB1-induced cell death. Hence, dysregulated autophagic pathways may be also involved in the development of vitiligo.

In addition, in patients with active nonsegmental vitiligo, HMGB1 plasma levels are increased compared with healthy controls and even HMGB1 expression in vitiligo lesional skin tissues is higher than in nonlesional skin. Therefore, HMGB1-induced melanocytic apoptosis may be pivotal in vitiligo. External stimuli (e.g., oxidative stress and UV irradiation) may trigger HMGB1 release by keratinocytes; therefore, an increased expression of HMGB1 is involved in vitiligo pathogenesis (28).

**References.**


