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Immunohistochemical Expression of c-kit Receptor (CD117) in Two Pigmentary Disorders

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Abstract

Stem Cell Factor (SCF) is generally thought to be an important regulator of melanocyte and mast cell survival, migration and proliferation. The c-kit receptor (CD117) may play a role in many skin disorders with dyspigmentation. The aim of this study was to assess the immunohistochemical expression of CD117 in some pigmentary disorders such as melasma and vitiligo. The study included a total of 40 patients were divided into two groups; melasma group: included (20) patients and vitiligo group: included (20) patients, in addition to a control group which included (20) healthy skin specimens were obtained during plastic operations. The severity of melasma and vitiligo skin lesions were measured by modified melasma and area severity index (MASI) and vitiligo area severity index (VASI) scores respectively. Tissue biopsies were taken from all the participant and stained by haematoxylin and eosin (H and E) and immunohistochemical staining for c-kit antibody. Normal skin: The intensity of c-kit expression was mild to moderate positivity. Melasma: The intensity of c-kit expression showed significant increase when its expression was compared with control group. There was statistically significant positive correlation between the intensity of c-kit expression in both melasma groups when compared with modified MASI score. Vitiligo: c-kit expression was negative in the epidermis. There was significant decrease in the intensity of c-kit expression in vitiligo when compared with control group. There was statistically significant negative correlation between the intensity of c-kit expression in both vitiligo groups when compared with VASI score. We concluded that c-kit may play a role in the pathogenesis of some dyspigmentary disorders as melasma and vitiligo. It could be used as a marker for melasma severity. So, c-kit may be considered the main target in the treatment of these diseases.

Introduction

CD117 (c-kit) is a transmembrane receptor tyrosine kinase that binds stem cell factor (SCF) [1]. c-kit is encoded by the kit proto-oncogene, localized to human chromosome 4 and to mouse chromosome 5 [2]. The c-kit/SCF interaction is critical for the survival and development of stem cells involved in hematopoiesis in pancreas development and in melanogenesis [3-5]. CD117 is a growth factor for melanocyte survival, migration and proliferation [6].

Melasma is a common acquired symmetrical hypermelanosis characterized by irregular light-brown to grey–brown macules and patches on sun-exposed areas of the skin [7]. The pathogenesis of melasma is not yet fully understood. It has been reported that paracrine linkages among keratinocytes, fibroblasts and melanocytes within the skin play important roles in regulating epidermal melanization in response to various stimuli [8-10]. The upregulation of such networks is intrinsically involved in the stimulation of melanocyte function in vivo in several epidermal hyperpigmentary disorders [11]. Lesional melasma skin showed more prominent solar elastosis compared with normal skin, suggesting that melasma may develop by activation of melanocytes overlying dermal changes caused by solar radiation [12,13].

Vitiligo is an acquired, idiopathic pigmentary disorder characterized by circumscribed depigmented white macules and patches surrounded by a normal or a hyperpigmented border [14]. The etiological basis of hypopigmentation in vitiligo is not completely understood; several mechanisms including apoptosis or functional impairment of melanocytes have been proposed [15,16]. The c-kit serves to maintain within the epidermis by attracting SCF, in the context of this important interaction between c-kit and SCF in stabilizing the melanocytes within the epidermis [17]. So, the aim of this study was to assess the immunohistochemical expression of CD117 in some pigmentary disorders such as melasma and vitiligo.

Patients and Methods

The present study was carried out on 40 patients with two pigmentary disorders. They were selected from the Outpatient Clinic of Dermatology and Venereology Department, Tanta University Hospitals. The studied groups were classified into:

Melasma group included 20 patients with melasma, diagnosed on the basis of wood's light and typical histopathological examination. All persons were females with different age groups. The severity of skin lesions was assessed by modified melasma and area severity index (MASI) score [18]. Vitiligo group included 20 patients with vitiligo, diagnosed on the basis of typical clinical and histopathological examination. All vitiligo patients were stable (grade 0 vitiligo disease activity score) [19]. The severity of skin lesions was assessed by vitiligo area severity index (VASI) score [20]. In addition a control group

including 20 healthy skin specimens obtained during plastic surgery from similar sites of the lesional tissue.

Exclusion criteria

Patients who received topical or systemic treatment in the past 6 weeks, prior to the incorporation in this study and patients who have other dermatological or systemic diseases.

After informed consent, 2 mm punch biopsies were taken from lesional skin of both melasma and vitiligo, skin biopsy from lesional melasma was taken away from the center of the face to avoid development of scar or disfigurement and skin biopsy from lesional vitiligo was obtained from photo exposed areas, all biopsies were formalin fixed and paraffin embedded. Haematoxylin and eosin stained specimens were examined for re-evaluation and confirmation of clinical diagnosis.

Immunohistochemical staining: Sections from formalin fixed paraffin embedded tissue blocks were cut at 5 μm thickness, deparaffinized in xylene, decreasing grades of ethanol and incubated with phosphate buffered saline containing 5% normal goat serum. Microwave antigen retrieval was done in citrate buffer twice for 20 minutes. Background staining was done using normal serum and hydrogen peroxide was used for blocking endogenous peroxidase. The detection system was the avidin biotin. Subsequently immunostaining with anti CD-117 rabbit polyclonal antibodies (Labvision Cat.# RB-9038-P, Fremont, California, USA) as primary ready to use antibodies. Color development was done using diaminobenzedin and Meyer's hematoxylin as counter staining. Negative control was done by omitting the primary antibody and the positive control was gastrointestinal stromal tumor. Cytoplasmic faint brown expression was considered positive reaction and melanin appeared as coarse brown pigmentation. The intensity of positive staining with the c-kit was graded as: Negative (zero): <25% of cells showed positive staining. Mild (+1): 25-<50% of cells showed positive staining. Moderate (+2): 50-75% of cells showed positive staining. Strong (+3): >75% of cells showed positive staining [21].

Statistical Analysis

All data obtained were transferred to the statistical package for the social sciences version 15 (IBM Co., New York, USA) for analysis. Data were summarized using mean, standard deviation (mean ± SD) and student's t-test. Comparison between groups were made by using X^2-test and Fisher's exact test for quantitative variables. Statistical significance was determined at a level of $p \leq 0.05$.

Results

Clinical results

Clinical results were illustrated in (Tables 1 and 2).

Histopathological results (H&E)

Normal skin: Normal count of melanocytes at the basal cell layer (Figure 1a).

Melasma: In ten cases (50%), epidermal type of melasma, the lesional skin biopsies showed increase in the number of melanocytes with increased melanization and condensation of melanin granules in the melanophores in the basal layers of the skin (Figure 1b). In ten cases (50%), mixed type of melasma, the lesional skin biopsies showed pigmented melanin granules and increased number of melanophages in the dermis; in addition to increased pigmentation in the epidermis (Figure 1c). All patients revealed moderate to severe solar elastosis (flattening of the rete redges with mild focal elastotic changes) helps in differentiation between normal and affected skin.

Vitiligo: Histopathological examination revealed degeneration of melanocytes was confined to the basal layer of the epidermis, acanthosis, and hyperkeratosis. (Figure 1d).

Immunohistochemical results for c-kit protein

Normal skin: The intensity of c-kit expression was mild to moderate positivity in basal cell layer (Figure 2b).

Melasma: The intensity of c-kit expression was moderate to strong diffuse cytoplasmic expression in the epidermis (Figures 2a and 3a). The intensity of c-kit expression showed significant increase when its expression was compared with control group [P value 0.006] (Table 3). There was statistically significant positive correlation in the intensity of c-kit expression in mild/moderate and severe melasma groups when compared with modified MASI score [p value 0.010, r value 0.362 and p value 0.013, r value 0. 421 respectively] (Table 4).

Vitiligo: c-kit expression was negative in the epidermis. One case only showed mild positivity (Figures 2c and 3b). There was significant decrease in the intensity of c-kit expression in vitiligo when compared with control [P value 0.004] (Table 3). There was statistically significant negative correlation in the intensity of c-kit expression in mild/moderate and severe vitiligo groups when compared with VASI score [p value 0.042, r value-0.269 and p value 0.037, r value-0.288 respectively] (Table 4).

Discussion

In mammalian skin, SCF is generally thought to be an important regulator of melanocyte and mast cell survival, migration and proliferation. CD117 may play a role in many skin disorders with dyspigmentation [22]. The major cell types that comprise the skin layers, including keratinocytes, fibroblasts, melanocytes and endothelial cells within the skin play important roles in regulating epidermal melanization in response to various stimuli, including endothelin-1, SCF, and c-kit [23]. The c-kit receptor is a proto-oncogene encoding CD117 that naturally binds SCF. It is also known as ligand mast cell growth factor. In response to various stimuli, keratinocytes secrete cytokines as SCF and c-kit so the levels of SCF secreted and c-kit increases in skin hyperpigmentation as melasma [24].

	Melasma group (NO=20)	Vitiligo group (NO=20)	Healthy controls (NO=20)	X^2 or t-test	p value
Average age (year, mean ± SD)	33.3 ± 6.3	27.7 ± 12.99	28.00 ± 11.61	1.635	0.099
Male/Female	0/20	14/6	13/7	1.203	0.120
Duration (year, mean ± SD)	3.10 ± 0.52	4.73 ± 4.47	-	0.635	0.523
Clinical types	Malar/centrofacial9/11	Focal/generalized/acrofacial 2/15/3	-	2.325	0.042*
With/without family history +ve/-ve	13/7	3/17	-	10.42	0.017*

Table 1: Clinical data of studied patients and controls.

		N	%
Modified MASI score	Mild/Moderate	8	40%
	Severe	12	60%
	Total	20	100
VASI score	Mild/Moderate	5	25%
	Severe	15	75%
	Total	20	100

Table 2: Clinical severity scores of the studied groups.
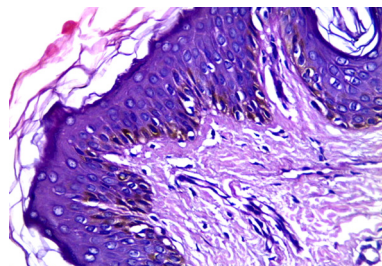

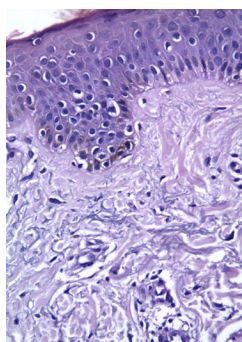**Figure 1a:** Normal skin; normal count of melanocytes at the basal cell layer (H&E×200).

Figure 1b: Epidermal melasma; showing increased number of melanocytes and melanophores in the basal layers of the epidermis (H&E×400).
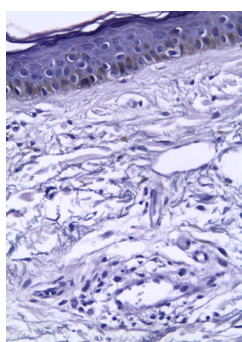

Figure 1c: Mixed melasma; showing increased number of melanocytes and melanophages in the epidermal and dermal layers of the skin (H&E×400).

Figure 1d: Vitiligo; showing complete absence of melanocytes in the basal cell layer with acanthosis and hyperkeratosis. (H&E×200).
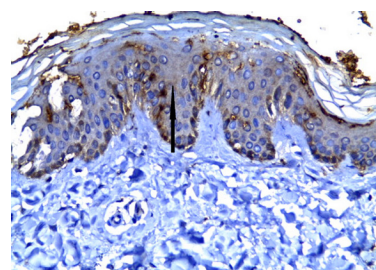
Figure 2a: Epidermal melasma; showing moderate (+2) cytoplasmic expression of c-kit mainly in the basal cell layer of the epidermis [arrow] (Strep-avidin biotin ×400).
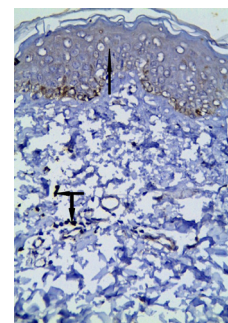
Figure 2b: Normal skin; showing mild (+1) expression of c-kit in the basal layer of epidermis [arrow] few positive cells in the dermis most probably mast cells [arrows] (Streptavidin-biotin ×400).
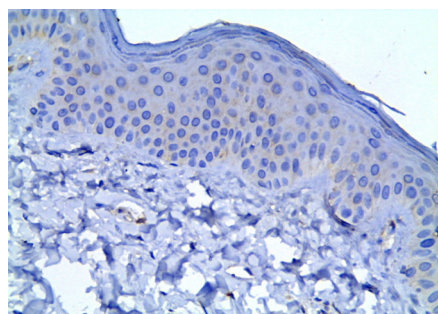
Figure 2c: Vitiligo; showing negative expression of c-kit (Streptavidin-biotin ×400).

In the present study, the expression of c-kit in normal skin was observed as homogenous brown stain in the cell membrane/cytoplasm of neural crest derived melanocytes and mast cells, where melanocytes were identified by their dendritic appearance and location in the basal cell layer of the epidermis. Lesional melasma skin showed statistically significant diffuse cytoplasmic expression of c-kit protein
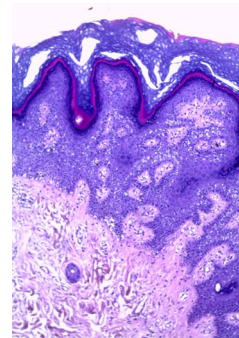
Grade	Intensity of c-kit	Group		
		Melasma=20	Vitiligo=20	Control=20
0	N	0	19	0
	%	0	95	0
+1	N	0	1	15
	%	0	5	75
+2	N	8	0	5
	%	80	0	25
+3	N	12	0	0
	%	20	0	0
Total	N	20	20	20
	%	100	100	100
Melasma and control	X^2	4.336		
	p	0.006*		
Vitiligo and control	X^2	6.247		
	p	0.004*		

P value ≤ 0.05 (significant difference)

Table 3: Intensity of c-kit expression in the studied groups.

immunohistochemical data of melasma	MASI Scoring	
	Mild/Moderate	Severe
r.	0.362	0.421
p. value	0.010	0.013*
immunohistochemical data of vitiligo	VASI scoring	8
	Mild/Moderate	Severe
r.	-0.269	-0.288
p. value	0.042	0.037*

P value ≤ 0.05 (significant difference)

Table 4: Correlation between the intensity of c-kit expression in melasma groups regarding to modified MASI score and vitiligo groups regarding to VASI score.

Figure 3a: Mixed melasma; showing strong (+3) cytoplasmic expression of c-kit mainly in both epidermis and dermis of the skin [arrows] (Strep-avidin biotin ×400).
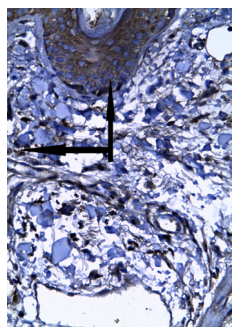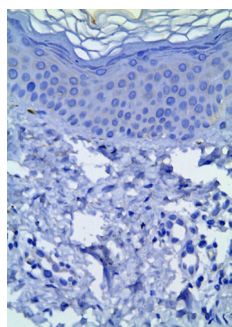

Figure 3b: Vitiligo; showing negative expression of c-kit in the epidermis (Streptavidin-biotin ×400).

with different intensities in all types of melasma. This result agreed with that reported by Kang et al. [23] which showed that SCF and c-kit were significantly increased in epidermis and dermis throughout the melasma lesions. This result suggests that SCF in the dermis may play a role in the development of melasma. It is possible that the inflammation in the dermis from the accumulated Ultraviolet Rays (UVR) may be associated with activation of fibroblasts and melanocytes leading to their increased proliferation and melanogenesis respectively [24]. These findings also were similar to another study, they were surprised by the unexpected evidence of damage to basal membrane, which could facilitate the fall or the migration of active melanocytes and melanin into the dermis allowing the constant hyperpigmentation in melasma [25]. The increased solar elastosis in melasma skin may suggest that the process of solar damage to the dermis might cause activation of fibroblasts to secrete melanogenic cytokines to increase SCF and c-kit, as exposure to UVR is one of the major aetiological factors in melasma. Finding abnormalities in the grade of elastotic material and mast cells infiltrate could be a key factor involved in its pathogenesis [23,26]. In response to various stimuli, human keratinocytes secrete various cytokines that serve as mitogens or melanogens for human melanocytes, including endothelin-1, SCF, fibroblast growth factor and α-melanocyte stimulating hormone. Human fibroblasts secrete several melanogenic cytokines, such as SCF and hepatocyte growth factor, which suggest the possibility that overexpression of these cytokines by dermal fibroblasts may activate melanocytes in the overlying epidermis in several epidermal hyperpigmentary disorders [23]. In the current study, there was positive correlation between the intensity of c-kit expression according to modified MASI score in both melasma groups suggesting its important role in the severity and the pathogenesis of the disease.

In the present study, lesional vitiligo skin showed significant decrease in the intensity of c-kit expression when compared with control. This result agreed with Dippel et al. [27] which conducted that melanocytes were found in uninvolved areas and absent in lesional skin. The study done by Kitamura et al. [28], showed that the expression of c-kit was significantly decreased at the edge of the lesional epidermis of vitiligo compared with the non-lesional epidermis. In the center of the lesion, there was complete loss of melanocytes expressing kit protein. They observed that the reduction of CD117 and its downstream targets [including Microphthalmia-Associated Transcription Factor (MITF)] by melanocytes might be associated with dysfunction or loss of melanocytes in vitiliginous epidermis. It is known that CD117 and MITF-M show complex interactions; CD117 signaling modulates MITF-M activity and stability in melanocytes, and MITF-M in turn is needed for the maintenance of CD117 expression on melanoblasts [29]. Another immunohistochemistry-based study demonstrated significantly lower expression of SCF and endothelin-1 in lesional skin compared with perilesional or nonlesional skin in patients with vitiligo [30]. Previous authors implicated decreased SCF expression by keratinocytes of vitiliginous skin to be responsible for apoptosis and loss of melanocytes [16]. The reason for the reduction in c-kit expression in vitiligo is not fully known. It has been suggested that mutations of the mi gene that might regulate c-kit expression result in melanocyte loss in mice. In particular mutant (mi vit) the pigmentation is normal at birth but there is progressive loss of melanocytes with age and has been proposed as a model for vitiligo [31]. The level of the c-kit/ mast cell growth factor ligand could also be important and it is possible that in vitiligo the production of this ligand is defective [32]. Abnormal expression or loss of the c-kit protein on epidermal melanocytes might explain the defective expansion and distribution of the pigment-

bearing cells in this disorder [33]. In the current study one case with vitiligo showed mild positivity of c-kit expression. This result may be due to that biopsy might be taken near the edge of the lesion or due to the effect of previous treatment although stopped 6 weeks a part. The total loss of melanocytes in vitiligo observed in this study supports the concept that depigmentation is the result of a melanocytotoxic event [33], propably related to immunological mechanisms and recent onset of the disease. CD117 is considered to be a candidate gene for vitiligo but more studies are needed to find its exact association to vitiligo onset, in this opinion as optimal utility for future therapeutic targets in the pathogenesis of vitiligo [34]. In the current study, there was significant negative correlation between the intensity of c-kit expression according to VASI score in both vitiligo groups suggesting its important role in the severity and the pathogenesis of the disease.

We concluded that c-kit may play a role in the pathogenesis of some dyspigmentary disorders such as melasma and vitiligo and it could be used as a marker for melasma and vitiligo severity. So, c-kit could be considered the main target in the treatment of these diseases. Up regulation [in vitiligo] or down regulation [in melasma] of this receptor may open the way for a further therapeutic approach.

Further studies might be done on hyper and hypopigmented disorders, before and after treatment to evaluate the use of c-kit as a marker for assessment of the prognosis of such lesions.

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