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Original Article

Follicular leukoderma is an early step in the course of ordinary vitiligo: A new hypothesis

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Abstract

Background Hair follicle is the primary target of many skin diseases. Vitiligo is a well-known autoimmune disease where the basal melanocytes of epidermis are attacked by the immunological reactions. Recently we had shown that the ordinary vitiligo might start initially in the hair follicles.

Methods Ninety patients with vitiligo having follicular leukoderma were involved during the period from 2014-21. Clinical and Wood's lamp examination were done to confirm vitiligo and exclude other hypopigmented diseases. Also, Skin biopsies for 10 cases with white follicular lesions and black hair were performed and stained with Haematoxylin-Eosin and immunohistochemical stains (HMB-45 and melan A) to evaluate histopathological changes.

Results 90 patients were analysed. Their ages ranged from 4 - 40 years with a mean of 20±4 years. Clinical and Wood's lamp examination showed that all patients had follicular white minute macules in early course of the disease that gradually coalesced into larger white macules and patches at the same sites. Histopathological examination including using immunohistochemical stain showed absent melanocytes in the hair follicle infundibulum and the adjacent basal layer of epidermis, perifollicular lymphocytic infiltrates and areas of focal invasion of follicular epithelium and the surrounding epidermis. In addition, superficial and to a lesser extent deep perivascular lymphocytic infiltrates were observed.

Conclusion Ordinary vitiligo initially starts as follicular leukoderma without necessarily causing leukotrichia, then these macules coalesce into larger vitiliginous patches.

Key words
Follicular leukoderma, vitiligo, hypothesis.

Introduction

Vitiligo is a well-known autoimmune disease where epidermal melanocytes are attacked by immunological reactions. Although the etiopathogenesis of vitiligo is still not well elucidated, many studies considered autoimmune theory singly or in combination with other mechanisms including cytotoxic and melanocytorrhagy leads to melanocytic loss.[1-5]

The current autoimmune theory suggests that a genetic susceptibility in addition to some environmental triggers leads to autoimmune distraction of melanocytes and causes depigmentation. For instance, genetic alteration of many genes involved in innate and adaptive immune regulation, pigment-producing enzymes (tyrosinase) and other associated proteins were identified to be associated with the disease development. Recently, three single nucleotide

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polymorphisms (SNPs) in *TYR* gene region showed significant genome-wide association with vitiligo; the strongest associations were with rs1847134 and rs1393350 SNPs. Other SNPs involving *IL2A* gene which encodes interleukin-2–receptor alpha chain on chromosome 10, showed significant genome-wide association with vitiligo. Additionally, variants of this gene were also found in other autoimmune disorders such as rheumatoid arthritis, type 1 diabetes, SLE and Grave's disease supporting a possible shared autoimmune etiology. Other immune regulatory gene SNPs were also documented to be significantly correlated with generalized vitiligo including MHC complex molecules class I (HLA-A) and II (HLA-DRb1).[6]

Furthermore, cytokines have a complex interplay role in vitiligo pathogenesis. Vitiligo is Th1 mediated disease and the expression of several cytokines are significantly increased in vitiligo lesions such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and IL-10.[7] IL-17 also has a role in vitiligo pathogenesis; it activates the expression of other cytokines such as IL-1, IL-6 and TNF-α. Levels of IL-17 in serum and lesional skin of 30 vitiligo patients showed a significant increase in IL-17 in vitiligo patients and with extent and duration of the disease.[8] Regarding the role of cell- mediated immunity, Dwivedi *et al*. found a significant increase in CD8+ T-cell counts in vitiligo patients as compared with the controls while CD4+/CD8+ ratio was significantly decreased. Also, they found a significant decrease in Treg cell percentage and counts in active vitiligo patients compared to normal and stable vitiligo. They suggested that an imbalance of CD4+/CD8+ ratio and natural Tregs in frequency and function might be involved in the T-cell mediated pathogenesis of vitiligo and its progression.[9]

It is well known that the structure of hair follicles consists of permanent upper segment part which includes the infundibulum, isthmus and the bulge area, while the lower hair follicle segment below the bulge area, including the bulb part, involutes during the telogen phase of the hair growth cycle.[10] The hair follicles are considered as a reservoir for melanocytes and as has been shown, there are many stem cells in the hair follicles especially in the permanent area of hair follicles so-called bulge area and the outer root sheath. These stem cells are melanocytes stem cells, epithelial stem cells and neural crest stem cells, but the most important one is melanocytes stem cells which are closely related to epithelial stem cells. During the repigmentation process, these stem cells proliferate, differentiate and move along the outer root sheath to reach the basal layer of the epidermis.[11,12]

Many case reports in medical literature described a new variant of vitiligo so- called follicular vitiligo.[13,14] It involves the hair follicle melanocytes and presents as grayness of body hair, scalp and beard areas. Surprisingly, these case reports did not mention specifically where the site of melanocytic loss takes place. In fact, it affects hair matrix melanocytes rather than other area, hence graying of hair occurs. Recently, Sharquie *et al*. described a new hypothesis about follicular vitiligo where the loss of melanocytes involves the fixed segment of hair follicle mainly the infundibulum and appears as tiny follicular depigmented macules. Subsequently, these follicular leukoderma would coalesce together to form ordinary patches of vitiligo.[15,16] Furthermore, repigmentation of vitiligo and burn leukoderma, usually starts at follicular hair orifices and then spread to peripheral areas. So, pigment loss and gain begin at the hair follicle with differences in the segment involved in each process; the infundibulum in the former and the bulge area in

the latter.[16-18]

In general, histopathology of vitiligo shows loss of melanocytes, epidermal vacuolization, epidermal and dermal T-cell inflammatory infiltrates, increased number of Langerhans' cells, absent or migrating melanocytes in outer root sheath, absence of Merkel cells and neural alterations.[19-21] However, limited studies showed the histological feature of follicular leukoderma in which the hair color is not affected by the disease damaging process.[22]

The objective of the present work is to support the hypothesis that ordinary vitiligo starts primarily and initially as follicular leukoderma then expands and coalesces to involve the surrounding interfollicular skin.

Patients and method

This is a case series descriptive and histopathological study where ninety patients with ordinary vitiligo having follicular leukoderma were collected during the period from 2014to2021. All demographic features were recorded. Full history and clinical examination including wood's lamp (365nm) examination were carried out. Skin biopsies from 10 cases with white follicular lesions and black hair were performed, processed and stained with Haematoxylin-Eosin stain and immunohistochemical stain (HMB 45 and Melan A). Patients with Segmental vitiligo were excluded from this study. Formal consent was taken from patients or their parents after discussing the nature of the study.

Table 1

Sex	Male	61	67.8%
	Female	29	32.2%
Age (years)	range	4-40	
	Mean ± SD	20 ± 4	
Total		90	100%

Results

The clinical data of 90 patients with follicular vitiligo were analyzed, their ages ranged from 4-40 years with a mean and SD of 20±4 years. 61 (67.8%) patients were males and 29 (32.2%) were females **(Table 1)**. Clinical and wood's lamp examination showed that all patients had follicular white minute macules. On close inspection, we noticed that these small follicular leukodermic spots gradually coalesced into larger white macules and patches in the same areas **(Figure 1)**.

Histopathological examination showed absent melanocytes in follicular infundibulum in 60% of patients, while 40% showed reduced melanocytes density using immunohistochemical staining. Reduced and/ or

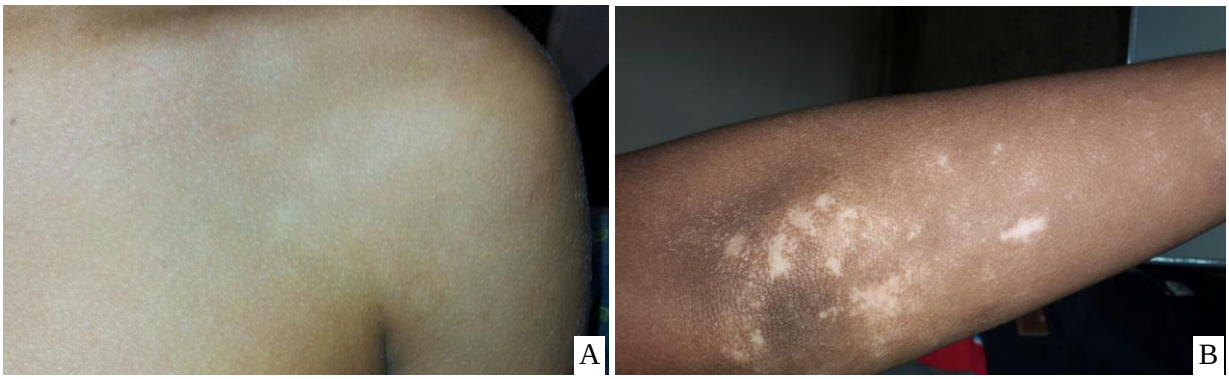

Figure 1 Follicular leukoderma (A) A 6 –years old male with follicular leukoderma coalescing into early hypopigmented patches of ordinary vitiligo involving the upper truck and shoulder. (B) A 10 –years old male patient with follicular leukoderma coalescing into completely depigmented patches involving the forearm.

Table 2 Histopathological findings of ten patients with follicular leukoderma.

Histopathological features	*Severity*	*No.*	*Percent*
Perivascular infiltrate	Superficial	7	70%
	Superficial and deep	3	30%
Perifollicular infiltrate (infundibulum and upper isthmus)	Mild	2	20%
	Moderate	7	70%
	Severe	1	10%
Melanophages	Present	3	30%
Follicular melanocytes	Reduced	4	40%
	Absent	6	60%
Basal epidermal melanocytes	Reduced ± focal loss	8	80%
	Diffuse loss	2	20%

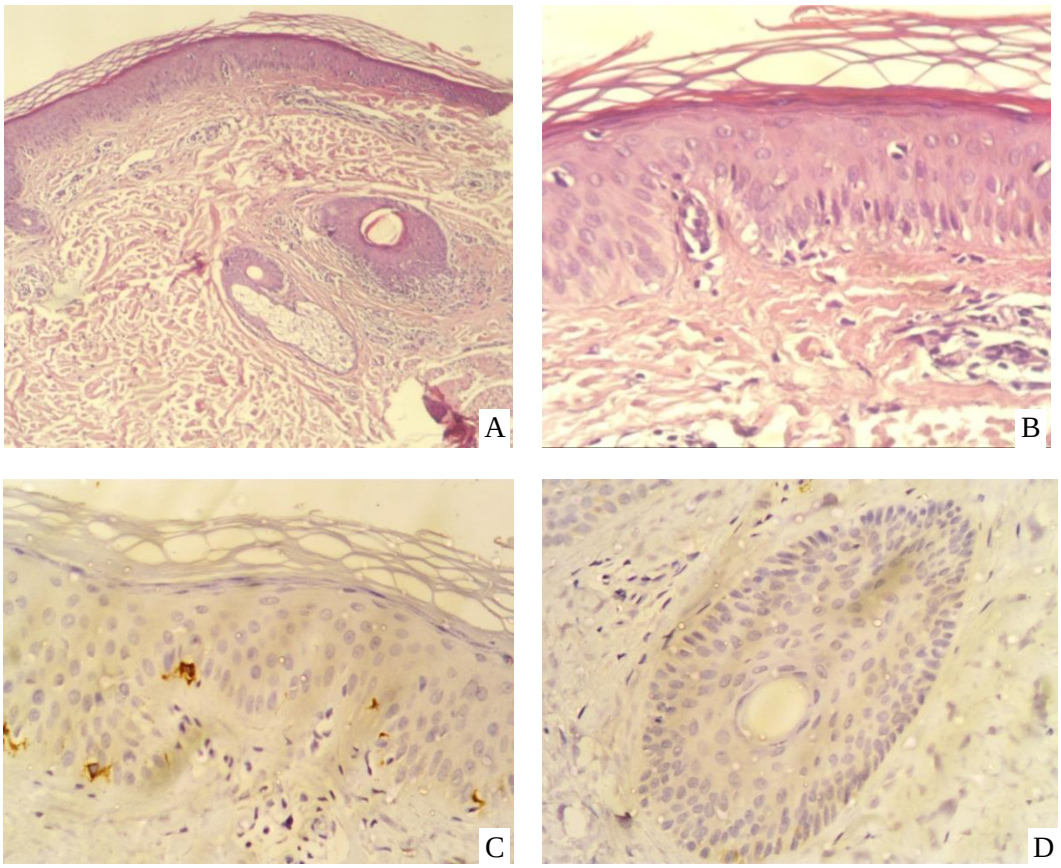

Figure (2) Histopathology of a 10-year-old female patient with follicular leukoderma and generalized vitiligo showing (A) moderate perivascular and severe perifollicular infiltrate with invasion at x 10. (B) Mild interface infiltrate, exocytosis, focal vacuolar degeneration, x40. (C) Preserved epidermal melanocytes using melan A stain,x40. (D) Complete follicular melanocytic loss,x40.

focal loss of melanocytes in the interfollicular basal epidermal layer has been seen in 80% and diffuse loss in 20% of patients. In addition, moderate perifollicular lymphocytic infiltrate around the infundibulum and the upper isthmus region of the hair follicle were observed in 70% of patients associated with focal lymphocytic invasion of the follicular epithelium. The epidermis was also affected by the inflammatory reaction in the form of lymphocytic exocytosis, focal basal layer vacuolar degeneration with lymphocytic invasion and dermal melanophages deposition. Also, mild to moderate dermal lymphocytic infiltrate mainly superficial (70%)

and to a lesser extent deep perivascular infiltrate or both (30%) was also detected **(Table 2, Figure 2)**.

Discussion

There are case reports describing follicular vitiligo as the involvement of body hair by vitiligo leading into graying/ whitening of hair.[13,14] This observation is noticed in segmental vitiligo and along with disease progression in nonsegmental vitiligo. Sharquie *et al*. reported for the first time that ordinary vitiligo starts initially in the upper segment of hair follicles including infundibulum producing follicular leukodermic macules rather than affecting the hair matrix melanocytes,[15] in contrast, graying or whitening of hairs means that there is loss of melanocytes of hair matrix. This might verify that follicular vitiligo consists of two elements: either there is loss of melanocytes of upper stable part of hair follicle mainly in the infundibulum leading to follicular then to ordinary vitiligo or there is loss of hair matrix melanocytes causing graying of hair.[18]

The present work had defined the hypothesis that vitiligo starts initially in the upper part of hair follicles and over time progresses into ordinary vitiligo where there is loss of melanocytes of the basal layer of the epidermis.

We observed variable perifollicular lymphocytic infiltrate mostly of moderate density present in the majority of cases along the upper segment of hair follicle associated with focal areas of invasion. Additionally, perivascular lymphocytic infiltrate mostly in the superficial vessels and to a lesser extent in the deeper compartments were noticed. These findings are consistent with Tag El-Din *et al*. results in which skin biopsies were taken from stable and active vitiliginous patches and normal appearing skin of patients with vitiligo. Their results showed moderate perifollicular and perivascular infiltrate, mainly lymphocytic, around black hairs in 70% of vitiliginous patches and 50% of apparently normal skin in the active cases.[23] However, in the present work, we took biopsies from early follicular leukoderma rather than from ordinary vitiligo patches or apparently normal skin.

Also, we observed total loss of melanocytes in the upper hair follicle epithelium bearing black hair in the majority of patients (60%). This result is consistent with the findings reported by Anbar *et al*. in which DOPA oxidase and NKI/beteb staining have been used for detection of melanotic and amelanotic follicular melanocytes respectively. Regarding black hair bearing vitiliginous areas, they detected melanotic melanocytes in the hair follicles in only 6.7% of patients and amelanotic melanocytes in 70% of patients. Furthermore, they found a significant correlation between disease duration and the absence of melanotic melanocytes in the hair follicles with time.[22]

In the present work, we only used Melan A and HMB 45 to detect melanotic melanocytes. Comparatively and despite that all biopsies showed an obvious reduction in melanocytes density, a higher percentage of our patients still have melanotic melanocytes in hair follicles (40%) which could be attributed to shorter disease duration as well as early stages of the disease progression. On the other hand, the majority of patients showed reduced or only focal loss of melanocytes in interfollicular basal epidermis rather than diffuse loss.

All these pathological observations along with the clinical presentation suggest that the inflammatory process might start at the upper follicular segment then involve the adjacent basal layer of the epidermis proper.

So the question that might be asked is why

vitiligo starts initially as follicular leukoderma?

Hair follicles are suspended inside the dermis; hence; have more extensive surface area that is exposed to any surrounding pathological damaging process. In addition, hair follicles have their distinct blood supply. This might indicate that there would be more intense inflammatory reaction in the hair follicle in comparison with the neighboring basal layer of the epidermis whereas only one surface of basal keratinocytes is exposed to the dermis. Also, melanocytes located in the basal layer of the epidermis are broadly similar to those located in the basal layer of the hair follicle infundibulum regarding their level of cellular differentiation.[24] Thus, we can think that the antigenic stimulation would be more intense in hair follicles compared to that in epidermis; as the density of various antigens or receptors is more in the keratinocytes of the outer root sheath than those of the epidermis proper. Accordingly the immunological and pathological reaction would be more florid in the hair follicles.[25-27] This inflammatory reaction would present as minute macules forming follicular lesions first and then coalesce to form larger vitiliginous patches.[15]

The immunological and inflammatory damage might involve the bulb of the hair as seen in alopecia areata and gray hair in vitiligo[28] or involve the upper segment of the hair follicle as in lichen planopilaris.[29] Similarly, when there is hyperkeratosis of the skin, it is more obvious at the orifices of the hair follicles than the surrounding epidermis as seen in many cases of keratosis pilaris.[26]

Although the disease starts initially as follicular lesions, this change could be transient or microscopic and might not be observed clinically in all cases. So the question that could be raised again is why the follicular lesions could not be observed all the time clinically or by histopathology? The following points might give the answers:

1- The follicular lesions could be so minute that could be missed or not be easily seen.
2- The lesions could be transient with a rapid expansion into the actual epidermis.
3- The pathological changes could be so minimal that could not be observed on histopathological sections.
4- Involvement of the hair follicle could be missed during the sectioning process of the biopsy.

Hence, for the above reasons, the prevalence of actual follicular vitiligo among the general population cannot be determined among patients with ordinary vitiligo but could be observed infrequently.

Finally, what about body areas where there are no hair follicles like palms and lips? Here in these areas, the orifices of sweat glands or sebaceous glands may represent the orifice of hair follicles or at these sites, the basal layer of the epidermis might be initially attacked.[30,31] For this purpose, further histopathological evaluation studies involving different glabrous vitiliginous areas are needed.

Conclusion

We can conclude that ordinary vitiligo initially starts as follicular leukoderma without necessarily associated with leukotrichia, and then these lesions coalesce together into macules and patches of vitiligo.

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