ORIGINAL ARTICLE

Pityriasis versicolor: Histopathological study

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ABSTRACT

Background: Pityriasis versicolor (PV) is a superficial chronically recurring fungal infection which usually present with multiple oval to round patches or thin plaques with mild scale. There are different clinical patterns of pityriasis versicolor.

Objective: The aim of this work was to study the histopathological changes in the different clinical patterns of pityriasis versicolor and to compare melanocyte activity in pigmented and hypopigmented lesions with that in the normal skin of the same patient.

Patients and methods: This study was carried out on 35 patients with pityriasis versicolor. Scrapings from the lesions were examined microscopically using KOH 15%. Two biopsies were taken from the back of each patient; one from the diseased skin and the other from the normal skin. The first portion was prepared in paraffin block and stained by Haematoxylin and eosin, Periodic Acid-Schiff (PAS), and Fontana stain. The second portion was stained by DOPA-oxidase (tyrosinase) reaction.

Results: With H&E stain, the affected skin showed hyperkeratosis and a patchy perivascular inflammatory infiltrate which were more marked in the hyperpigmented than in the hypopigmented type. Using PAS stain, spores and hyphae were seen in the horny layer of all the hypopigmented and hyperpigmented pityriasis versicolor lesions. Fungal elements were more marked in the hyperpigmented type. Cases with post-pityriasis versicolor hypopigmentation showed no fungal elements. With Fontana stain, there was a decrease in the density of melanin pigment in keratinocytes in the hypopigmented cases, while the hyperpigmented lesions showed an increased density. DOPA reaction showed decreased density of melanin pigment in melanocytes of the hypopigmented cases compared to normal skin of the same patient. The reverse was found in the hyperpigmented cases.

Conclusion: H&E stain showed changes that were more marked in hyperpigmented lesions and may play a role in production of hyperpigmentation. DOPA reaction may indicate some sort of disturbance in melanin formation inside the melanocytes. Changes revealed by Fontana stain may be due to a decrease in melanin granules transfer to keratinocytes or non-replacement of the already shed melanin granules.

KEYWORDS: Pityriasis versicolor, histopathological findings, melanocyte activity, pigmented skin, hypopigmented skin, normal skin.

INTRODUCTION

Pityriasis versicolor (PV) is a superficial chronically recurring fungal infection which usually present with multiple oval to round patches or thin plaques with mild scale.¹ It is caused by yeasts of the Malassezia spp. genus commensal of the keratinized layers of the skin which under certain conditions not yet understood, becomes pathogenic determining the clinical manifestations of the disease.² Malassezia spp. are implicated in several mild, but recurrent cutaneous diseases, such as PV, Malassezia folliculitis, seborrhoeic dermatitis, and, with lesser frequency, a range of other dermatological disorders.³ Some studies showed that Malassezia globosa presents the main species implicated in the pathogenicity of

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PV⁴⁻⁸ and Malassezia furfur as the second agent of importance^{9,10} while Malassezia sympodialis were the most prevalent species isolated in other studies.^{11,12} PV in most cases represents a shift in the relationship between a human and his resident yeast flora. It is known that some Malassezia species more readily become mycelial, and have perhaps a slightly greater pathogenic potential.¹³ The highest incidence of PV was observed in young persons compared to other age groups¹⁴ and the occurrence of PV before puberty or after age 65 years is uncommon.¹⁵ The fungus is easily demonstrated in scrapings of the scales soaked in 10% KOH.¹⁶ histopathologically, the horny layer of PV lesions contains abundant fungi and the inflammatory response is usually minimal, although there may be slight hyperkeratosis, slight acanthosis, or a minimal perivascular superficial lymphocytic infiltrate.17

The aim of the work is to study the histopathological changes in pityriasis versicolor, and to compare melanocyte activity in various pigmented and hypopigmented lesions with that of the adjacent normal skin.

PATIENTS AND METHODS

This work included 35 patients with PV. The studied cases represented the different clinical patterns including pigmented, hypopigmented and post-PV hypopigmented lesions. The 35 cases were divided into three groups, according to the clinical type of the disease: group I which included 19 cases of hypopigmented PV, group II with 9 cases of hyperpigmented PV and group III with 7 cases with post PV hyperpigmentation.

The selected cases were subjected to clinical, mycological and histological studies. The clinical Study included history and clinical examination, noting onset, course, morphology and localization of the lesion.

Mycological study was done by Scrapings from the lesions and examining them microscopically using KOH 15%.

Skin surface biopsies were done using cyanoacrylate contact cement for quick direct examination after staining with PAS for histological study.¹⁸ After local anesthesia with xylocaine 2%, two biopsies were taken from the back of each patient. One specimen was taken from the diseased skin and the other from the adjacent normal skin. Each biopsy was divided into two portions:

The first portion was prepared in paraffin block and stained by:

- Haematoxylin and eosin (H & E) stain:¹⁹ Sections were stained in haematoxylin in a Jar for two minutes. Sections were washed well in running tap water 2-3 minutes. Excess stain was removed by decolorizing in 0.5-1% hydrochloric acid in 70% alcohol for a few seconds. The blue color of the haematoxylin was changed to red by the action of the acid. The blue color was regained and decolorization was stopped by washing in alkaline. Staining in 1 % aqueous eosin for 1-3 minutes was performed. Washing off surplus stain in water was done. Dehydration in alcohol, clearing in xylene and mounting in Canada balsam were performed.
- 2. Periodic Acid-Schiff (PAS):²⁰ Sections were brought to water. Oxidation for 5 minutes in 1% aqueous periodic acid was done. Washing in running water for 5 minutes and rinsing in distilled water were performed. Sections were placed in Schiff reagent for 20 minutes. Washing for 10 minutes in running water was done.

Counter-staining with haematoxylin was done. Dehydration in alcohol, clearing in xylene and mounting in Canada balsam were performed.

Fontana Method:²¹ Strong ammonia was add-3. ed drop by drop to 20 cm³ of 10 percent silver nitrate until only a slight trace of the precipitate remained, then 20 cm³ of distilled water was added. Paraffin sections were brought to water Sections were placed in Gram's iodine for 10 minutes. Sections were transformed to 3% sodium thiosulphate for 2 minutes. Sections were washed well in several changes of distilled water. Sections were left overnight in the silver solution, described above, in dark in a closed jar. Rinsing in distilled water was done. Fixation in 5% sodium thiosulphate for 2 minutes was done. Dehydration, cleaning and mounting in Canada balsam was performed.

The second portion was stained by DOPA-oxidase (tyrosinase) reaction:²² Cryostat frozen sections were cut. Sections were washed briefly in distilled water. Sections were placed in incubating solutions at 37C° for 45 minutes. DOPA solution was changed and incubation is continued for at least two hours. Washing in water was done. Dehydration in alcohol, clearing in xylene and mounting in Canada balsam were performed.

RESULTS

The present study included 35 PV cases, the studied cases included 21 males and 14 females and their ages ranged between 16 to 59 years. The duration of the condition varied between two weeks and six months, and the past history of recurrence was present in 22 cases.

Results of Mycological Examination:

Microscopical examination of scraping from the

Lesions Using KOH 15%: The scrapings were examined for the presence of spores and hyphae in the three groups of patients. All the cases in group I and were II KOH positive, while All the cases in group I were KOH negative.

Microscopical examination of the lesions by skin surface biopsy stained with PAS demonstrated spores and hyphae in both group I and group II but they were more abundant in group II (Fig. 1). Lesions of group III showed no spores or hyphae.

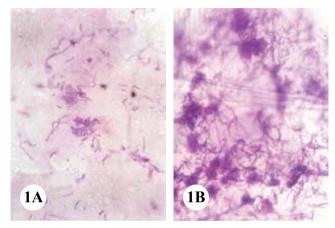


Fig. 1 Skin surface biopsy (PAS, X500). A: hypopigmented PV. B: hyperpigmented PV Spores and hyphae are seen. They are more dense in hyperpigmented than in hypopigmented type.

Results of the Histopathological Examination:

 Haematoxylin – Eosin Stain (H&E): Group I showed mild degree of hyperkeratosis in all the cases, mild acanthosis in 10 cases and mild patchy dermal inflammatory infiltrate composed mainly of lymphocytes in all the cases (Fig. 2A). In group II, five cases showed moderate hyperkeratosis and four cases showed marked hyperkeratosis. Mild acanthosis and moderate patchy dermal lymphocytic infiltrate were seen in all the cases. The infiltrate was mainly perivascular (Fig. 2B). The seven cases of group III were histologically within normal. Table 1 demonstrates the results of haematoxylin and eosin (H & E) stain.

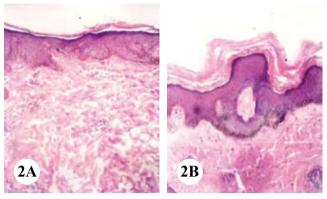


Fig. 2 (H&E, X200) A: hypopigmented PV showing mild hyperkeratosis, mild acanthosis and mild dermal inflammatory infiltrate. B: hyperpigmented PV showing moderate hyperkeratosis, mild acanthosis and moderate dermal inflammatory infiltrate.

 Table 1 Results of (H & E) stain of the studied cases compared to normal skin

Group	Hyperkeratosis	Acanthosis	Dermal infiltrate
Normal Skin	-	-	-
Group I	+	+(10)	+
		- (9)	
Group II	+++ (4)	+	++
	++ (5)		
Group III	-	-	-

+; Mild ++; Moderate +++; Marked -; absent

2. PAS stain: The 19 cases of group I and the nine cases of group II showed the short thick hyphae and the rounded spores of PV in the horny layer. The spores and hyphae are more numerous and more easily identified in the hyperpigmented than in the hypopigmented cases (Fig. 3). The seven cases of group III were devoid of fungal elements.

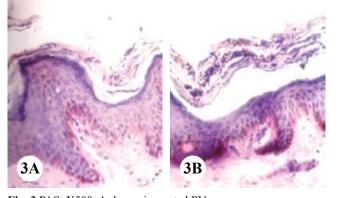


Fig. 3 PAS, X500: A: hypopigmented PV. B: hyperpigmented PV. Hyphae and spores are present in stratum corneum in both sections, but they are more in number in hyperpigmented than in hypopigmented type.

3. Fontana stain: In the 26 hypopigmented cases (the 19 cases of group I and the 7 cases of group III), Fontana stain showed that the density of melanin pigment in keratinocytes of the diseased skin is less than that of adjacent normal skin of every patient. In the nine hyperpigmented PV cases, there was an increased intensity of stain in keratinocytes of the diseased skin, compared to adjacent normal skin (Fig. 4).

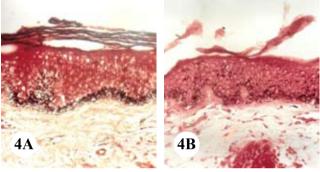


Fig. 4 (Fontana, X300). A: hyperpigmented PV. B: normal skin. There is an increased intensity of the stain in the keratinocytes of the diseased skin compared to normal skin.

4. **DOPA reaction:** In the 19 hypopigmented PV cases and the seven cases with post-PV hypopigmentation, DOPA reaction showed that the difference between the diseased skin and the normal skin was in the intensity of DOPA reaction in melanocytes. The color was relatively faint in the diseased skin compared to the adjacent normal skin (Fig. 5).

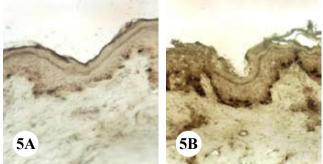


Fig. 5 (DOPA, X300). A: hypopigmented PV. B: normal skin. The intensity of DOPA reaction in melanocytes is less in the diseased skin compared to normal skin.

In the hyperpigmented PV cases, the intensity of DOPA reaction in melanocytes in the diseased skin was more, compared to normal skin. Table 2 shows the results of Fontana stain and DOPA reaction.

Table 2 Intensity of Fontana stain and DOPA reaction of thestudied cases compared to normal skin

Group	Fontana	DOPA
Normal Skin	++	++
Group I	+	+
Group II	+++	+++
Group III	+	+

+; Mild ++; Moderate +++; Marked

DISCUSSION

The purpose of this work was to study PV histologically and to compare melanocytes activity in the different morphological patterns of PV with that of the normal skin of the patient.

On (H&E) stain, both the hypopigmented and hyperpigmented PV lesions showed hyperkeratosis, but it was evidently present in the hyperpigmented lesions contrasting with the slight hyperkeratosis in the hypopigmented lesions. Similar findings were demonstrated by Galadari et al.²³ Furthermore, Karaoui et al found similar results.²⁴

The dermis showed a patchy perivascular lymphocytic inflammatory infiltrate. This infiltrate was more pronounced in the hyperpigmented than the hypopigmented lesions which showed only a low grade inflammatory cell infiltrate. These findings were similar to those obtained by Galadari et al.²³ However, the study of El-Gothamy el al²⁵ on hypopigmented PV revealed that the dermis in the hypopigmented lesions contained minimal inflammatory infiltrate without commenting on any tendency for perivascular or periadnexal location.²⁵ The cases with post PV hypopigmentation were within normal on (H&E) stain. This coincides with the findings of El-Gothamy et al.²⁵ Reported atypical histological findings of PV include absence of a granular layer, keratinocyte vacuolization, follicular plugging, dilated blood vessels, pigment incontinence and subepidermal hyalinization.²⁶ Effacement of rete ridges, dermal atrophy, elastolysis²⁷ and interface dermatitis have also been reported.²⁸

The spores and hyphae were more numerous in the hyperpigmented than hypopigmented lesions, a finding which was demonstrated in skin surface biopsy as well. This predominance of fungal elements in the horny layer of the hyperpigmented type was demonstrated as well by Galadari et al.²³

The more pronounced dermal infiltrate and fungal elements in the hyperpigmented PV suggested that these changes may have a role in production of hyperpigmentation. This could be due to the optical effects of the increased fungal elements as well as the hyperkeratosis in the horny layer. Furthermore, an increased melanocytes activity may also contribute .One may speculate that this increased activity is due to direct stimulation by cytokines derived from the infiltrating inflammatory cells.

The decrease in the density of melanin pigment inside the melanocytes of hypopigmented PV lesions and post-PV hypopigmentation compared with normal skin correlate with findings of El-Gothamy et al.²⁵ This could be considered as evidence that there is some sort of disturbance which affects the process of melanin formation inside the melanocytes.

Fontana stain showed also a decrease in the density of melanin pigment in keratinocytes in the abovementioned two groups (hypopigmented PV and post-PV hypopigmentation). This may indicate a decrease in melanin granules transfer by the melanocytes or non-replacement of the already shed melanin granules as no newly formed pigments could be produced by the melanocytes.

In the hyperpigmented PV lesions, DOPA reaction showed increased density of melanin pigment in the melanocytes compared with normal skin. Fontana also showed increased pigment in the keratinocytes. These findings indicate increased melanin formation and transfer.

The micro-environment of the skin, which prevents marked growth of the organism, may lead to the production of chemical mediator (dicarboxylic acids or yet unidentified ones) which cause disturbances in melanocytes function. Nazzaro-Porro and Passi²⁹ suggested that the hypopigmentation is due to dicarboxylic acids formed through oxidation, by malassezia enzyme system, of the unsaturated fatty acids normally present in the skin surface lipids.²⁹ El-Gothamy and Moneib³⁰ revealed that melanocytes in the hypopigmented lesions contained melanosomes which more or less of the same number as those of the normal adjacent areas. However, these melanosomes were immature, smaller, more rounded and light in color. They concluded that these donate poor melanization which also could be attributed to formation of dicarboxylic acids by malassezia.³⁰ A recently described tryptophan-dependent pigment metabolism of Malassezia furfur might be of importance in this context, as certain compounds possess biological activities providing an explanation for different symptoms of PV.³¹ Malassezin (characterized as agonist of the aryl hydrocarbon receptor from the yeast Malassezia furfur), for example, induces apoptosis in human melanocytes,³² and therefore may be responsible for depigmentation. Pityriacitrin, which absorbs UV radiation,

may be accountable for reduced UV sensitivity of the depigmented areas.³³ However, pigment production could not yet be induced in M. globosa which is currently regarded as a main causative agent of PV. It appears that M. globosa possesses homologues to most of the genes that are differentially expressed during pigment production in M. furfur.³⁴

In contrast to hypopigmented PV, the election microscopic observation made on hyperpigmented lesions showed that melanocytes were larger and melanosomes were hypertrophic and singly distributed.²⁴ However, it was suggested that the hyperpigmentation is most likely secondary to multiple factors; including the increased thickness of keratin layer, the presence of larger number of organisms, and the more prevalent lymphocytic infiltrate.²³ Furthermore, expression of a tryptophan aminotransferase could be shown in skin scrapings of lesions of PV by semiquantitative PCR³⁵ and it was suggested to play a role in the production of hyperpigmentation in PV.³⁴

CONCLUSION

The histopathological study of PV lesions using H&E stain showed hyperkeratosis and a patchy perivascular inflammatory infiltrate. These changes were more marked in hyperpigmented lesions and may play a role in production of hyperpigmentation. With PAS stain, fungal elements were more marked in the hyperpigmented type and this may be also related to hyperpigmentation. DOPA reaction showed decreased density of melanin pigment in melanocytes of the hypopigmented PV cases as well as the post PV hypopigmentation while the reverse was found in the hyperpigmented cases. This may indicate some sort of disturbance in melanin formation inside the melanocytes. The density of melanin pigment in keratinocytes decreased in the hypopigmented PV lesions and increased in the hyperpigmented ones as shown by Fontana stain and this may be due to a decrease in melanin granules transfer to keratinocytes or non-replacement of the already shed melanin granules.

Lastly, neither this work, nor other studies, even those using ultrastructure, could clearly explain the dyschromia in PV. However, the recently described tryptophan-dependent pigment metabolism of Malassezia furfur as well as the recently reported genome and secretory proteome of Malassezia globosa together with the examination of cytokines network in the different presentations of the disease may forge a path in the proper understanding of the dyschromia in PV.

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