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STUDY OF ONYCHOMYCOSIS AND INTERLEUKIN-10 IN PSORIATIC PATIENTS

By

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ABSTRACT

In patient with psoriasis who presents with a nail abnormality it may be clinically difficult to decide whether this is due to a psoriasis, onychomycosis, a combination of these or due to some other causes. Interleukin-10 (IL-10) has anti-inflammatory and immunosuppressive properties. Elevated IL-10 might contribute to an enhanced risk for development of skin superinfections found frequently in several dermatoses. The aim of this work is to study the role of lab. in diagnosis of onychomycosis in psoriatic patients and the relation between fungal infections and serum level of IL-10. Nail clippings from 31 patients under-anti-psoriasis therapy were subjected to microscopic examination and culture on sabauroud media with cyclohexamide and without cyclohexamide. Blood samples from patients and 10 healthy controls were collected and sera were separated and analysed for IL-10 determination by ELISA. Nail clippings form 18 samples (58.1%) had fungal growth. The most common isolated fungus was Candida albicans (38.9%) followed by Trichophyton rubrum (33.3%), Trichophyton mentagrophytes (16.7%) and Epidermophyton floccosum (11.1%). IL-10 was increased in patients than control (P= 0.0001). Higher level was observed in patients with fungal infection (mean 69.31 ± 50.11pg/ml) than in patients with no growth (67.26 ± 58.85 pg/ml). From this study it could be concluded that psoriatic patients had increase liability to onychomycosis which should be excluded by laboratory means prior to treatment of psoriatic nail with corticosteroid. Patients had increase serum level of IL-10 which may predispose to fungal infections by its immunosuppressive effect.

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INTRODUCTION

Onychomycosis is a persistant fungal infection of the toe nails or finger nails that is usually not painfull but is unsightly and can affect a patient's quality of life by interfering with foot wear [5]. The most common infections are those caused by dermatophytes. Part of the diagnostic challenge lies in distinguishing the mycotic lesions from those caused by cutaneous diseases such as psoriasis, eczema, dyshidrosis and contact dermatitis [7].

Proper management, therefore includes confirmation of fungal infection by potassium hydroxide slide preparation and culture [21].

Psoriasis is a common hyperproliferative and inflammatory skin disease with a prevalence of 0.5-3% [15]. There is a cascade of cellular and molecular events that produce psoriasis, primarily keratinocyte hyperplasia, altered differentiation and an angiogenic tissue reaction [16].

In psoriatic patients who present with nail abnormalities, it may be clinically difficult to decide whether this is due to psoriasis, onychomycosis, a combination of these or due to other cause. In addition, the prevalence of onychomycosis in patients with psoriasis has been controversy [8].

Some therapies for psoriatic nails such as corticosteroids may worsen concomitant onychomycosis. Also, fungal infection of the psoriatic nail unit might adversely affect the response to antipsoriatic therapy [12].

Over expression of proinflammatory type I cytokines has been demonstrated and is believed to be of pathogenic significance in psoriasis. Interleukin- 10 (IL- 10) is a type 2 cytokine with major influences on immunoregulation, inhibiting type 1 proinflammatory cytokine production. Patients on established psoriasis traditional therapies show increasing levels of IL-10 [2].

Abnormal cytokine response involving IL-l0 may be implicated in chronic fungal infections [20].

The aim of this work is to study the role of lab. in diagnosis of onychomycosis in psoriatic patients and the relation between fungal infections and serum level of IL- 10.

SUBJECTS AND METHODS

This study was carried on 31 patients (19 females and 12 males) and 10 healthy controls. All patients were under systemic antipsoriatic therapy including corticosteroid, PUVA and cyclosporine.

For a psoriatic patient to be included in the study clinical evidence of psoriasis at a site other than the nails was required.

Samples:

* Nail clippings from the affected nails were taken for mycological examination.

* From each patient and control subject 4 ml blood were taken and sera were separated and kept frozen at -20°C for interleukin 10 determination.

Methods:

A) Nail clippings were subjected to the following:

- Light microscopy examination by (KOH) preparation.

- Culture on sabouraud peptone-glucose agar with added cyclohexamide, chloramphenicol and gentamycin and on sabouraud's media without cyclohexamide.

- Positive culture was identified by lactophenol stain and if proved to be yeast-further identification was performed by API candida.

API Candida:*

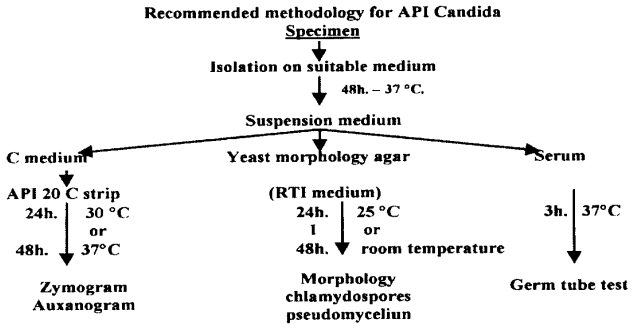
API candida is a standardized system for the identification in 24 - 48 hours of the yeasts most frequently encountered in clinical microbiology.

Principle:

The API Candida strip consists of 10 tubes containing dehydrated substrates, which enable the performance of 12 identification tests (Sugar acidification or enzymatic reactions).

The reaction produced during incubation is revealed by spontaneous colour changes.

After an incubation period of 24-48 hours at a temperature of 35°C-37°C, reading of the reactions is performed visually and identification is obtained by consulting the list of profiles at the end of this package insert.



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*Interleukin 10 (IL-10)***

The assay is based on a sandwich technique with a monoclonal IL-10 as the antibody against capture antibody and a second biotinylated monoclonal antibody as the detection antibody. This second antibody reacts with streptavidin peroxidase. The bound enzymatic activity is determined by addition of a chromogenic substrate (TMB) and by measuring the resulting coloured solution with a spectrophotometer. The concentration of the sample or the standard is proportional to the absorbance value measured.

Calculation of results: calculate the mean absorbance value of standards and samples. Determine the net absorbance by substracting the assay balnk absorbance from the mean absorbance value of each standard and sample. Draw standard curve by plotting the standard concentrations (IL-10 pg/L) on the horizontal axis versus the corresponding net absorbance values on the vertical axis.

RESULTS

This study was performed on 31 patients complaining of psoriasis attending the outpatients clinics of Dermatology and Venereology Department. They were 19 females and 12 males with age range 13 - 80 years (mean 43 ± 16.01). Also, 10 healthy controls of matched age and sex were added for serum interleukin- 10 level determination.

Nails culture revealed 18 positive cases (58.1%) and 13 negative cases (41.9%). Table 3 & Fig. 1.

Fungal growth in positive cases were Candida albicans in 7 cases (38.9%), Trichophyton rubrum in 6 cases (33.9%), Trichophyton mentagrophytes in 3 cases (16.7%) and Epidermophyton floccosum in 2 cases (11.1%) Table 4 & Fig. 2.

Photograph (1) shows Candida albicans identified by API Candida.

The duration of the disease was relatively longer (3.5 years) in patients with positive growth than cases with no growth (3 years).Table 5.

Regarding interleukin- 10 level comparison between patients and control, IL-l0 was increased in patients (mean 68.45 ± 53.009), more than control (11.3 ± 1.947) and this increase was statistically significant (p=0.0001). Table 6 & Fig. 3

In cases with positive growth IL-10 level was increased (69.31 ± 50.11) than cases with no growth (67.26 ± 58). However this increase was statistically insignificant (p=0.68). Table 7 & Fig.4

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Table (1): Age distribution of studied groups.

	Patients	Control	Test of significance
Age range	12 – 80	14 – 80	t = 0.18
Mean ± SD	43.32 ± 16.01	44.4 ± 17.68	p = 0.85

Table (2): Sex distribution of studied groups.

	Patients		Control		Test of significance
	No	%	No	%	
Male	12	33.7	5	50	$x^2 = 0.18$
Female	19	61.3	5	50	p = 0.52

Table (3): Culture of nails.

	No	%
No growth	13	41.9
Growth	18	58.1

Table (4): Type of fungus in growth

	No	%
Candida albicans	7	38.9
Trichophyton rubrum	6	33.3
Tricophyton mentagrophytes	3	16.7
Epidermophtyon floccosum	2	11.1
	18	100

Table (5): Duration of the disease according to presence of growth

	No growth (n = 13)	Growth (n = 18)
Mean ± SD	7.09 ± 9.21	9.5 ± 10.95
Median	3	3.5
SE	2.55	2.58
Z = 0.32 P = 0.74		

Table (6): Comparison between patients and control regarding Interleukin-10 (pg/ml).

	Patients (n = 31)	Control (n = 10)
Mean ± SD	68.45 ± 0.009	11.3 ± 1.947
Median	61	9
SE	8.95	0.416
Z = 4.71 P = 0.0001		

Table (7): Comparison between cases with positive and negative growth regarding Interleukin-110.

	Negative growth (n = 13)	Positive Growth (n = 18)
Mean ± SD	67.26 ± 58.85	69.31 ± 50.11
Median	50	50
SE	16.32	11.80
Z = 0.40 P = 0.68		

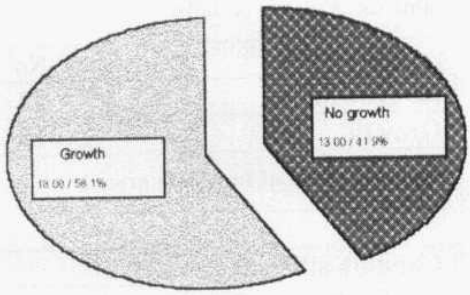
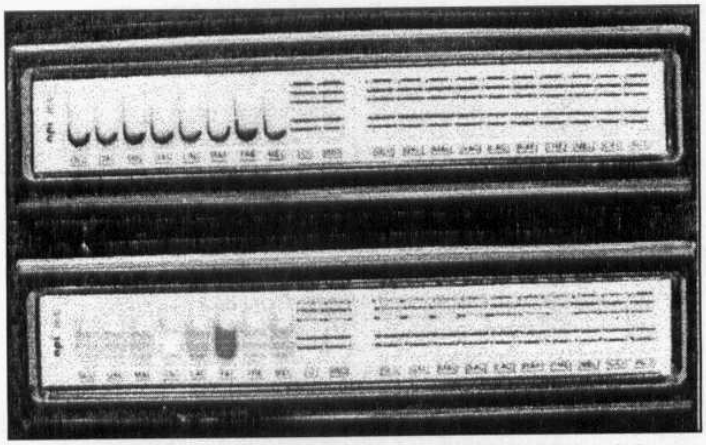


Fig. (1): Culture of nails among studied cases.



Photograph (1): Candida albicans identified by API Candida

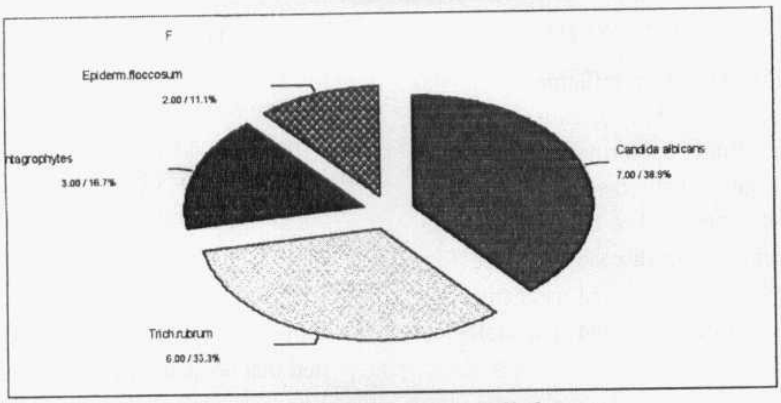


Fig. (2): Type of fungus among studied cases.

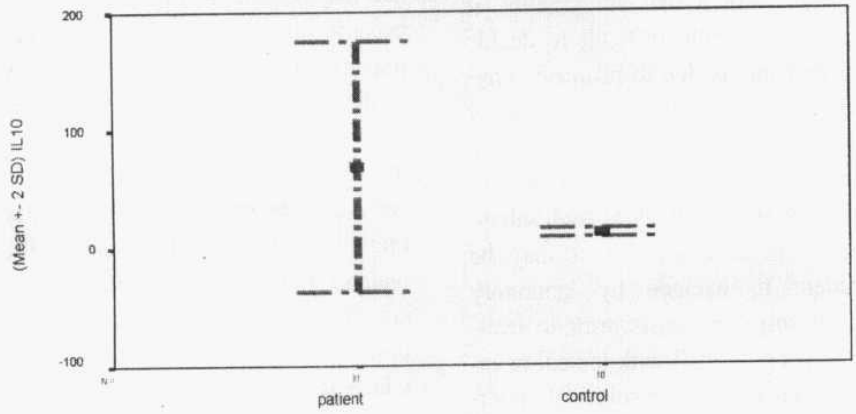


Fig. (3): IL10 among cases and control.

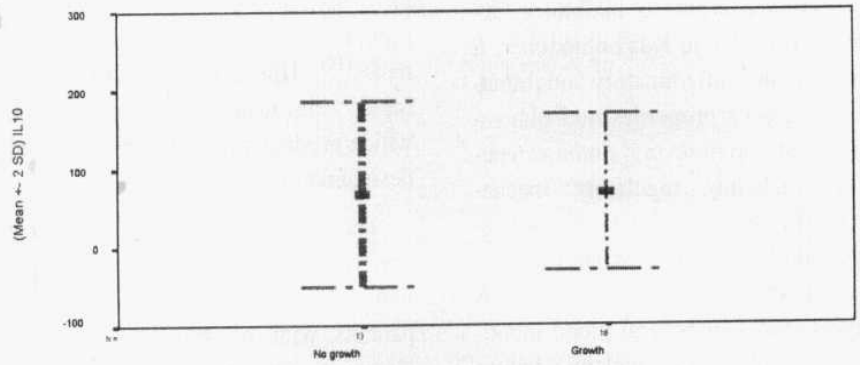


Fig. (4): il 10 among cases according to growth.

DISCUSSION

Psoriasis is an inflammatory papulosquamous skin disease associated with rapid epidermal proliferation. The cause of the disease is unknown, but it is believed to be genetically determined. The disease could be triggered by chemical and mechanical injuries, infections and psychological stress [17].

In patients with psoriasis who presents with a nail abnormality, it may be clinically difficult to decide whether this is due to psoriasis, onychomycosis, a combination of those or due to some other causes. The common features of psoriasis and onychomycosis are onycholysis and subungual hyperkeratosis. So, it may be prudent to exclude by laboratory means onychomycosis prior to treating a psoriatic nail with topical or intralessional corticosteroids [8]. Interleukin- 10 (IL-10) was first described as "cytokine synthesis inhibitory factor". It is a 35 - 40 Kda homodimer. It has both anti-inflammatory and immunosuppressive properties and plays a dominant role in several immune reactions including regulatory mechanisms in the skin. Interestingly, patients during established antipsoriatic therapy showed higher IL-10 mRNA expression of peripheral blood mononuclear cells than patients before therapy [2]. The aim of this work is to study the role of lab. in diagnosis of onychomycosis in psoriatic patients and the relation between fungal infections and level of interleukin- 10. From 31 patients included in this study 18 patients had fungal growth (58.1%). Table 3 & figure 1.

Similarly Gupta et al (1997) reported that 56% of patients with psoriasis had onychomycosis. An abnormal nail may develop in a psoriatic patient because of prior damage to that nail, impairment of peripheral blood flow or the psoriatic state [3]. These abnormalities could predispose to onychomycosis. Some antipsoriatic therapies such as methotrexate and cyclosporin might alter the immune state of the patients predisposing to onychomycosis. Thus both infection and psoriasis produce abnormal nails in psoriasis which is difficult to be differentiated on clinical bases only. However, there have been some reports that dermatophytes are uncommon in psoriatic nails [19]. This could be attributed to rapid outgrowth of psoriatic nails, which produce the rapid turnover, and desquamation of the nail unit [8].

The median duration of the disease was relatively longer in the patients with growth (3.5 years) than in patients with no growth (3 years). However, this increase in duration

was statistically insignificant (p=0.74). Table 5. The increase time of exposure of nails to psoriatic changes may increase susceptibility to fungal infection [8]. However, insignificant increase of duration in cases with positive culture could be explained by inaccuracy in the patients recall which lead to obscure the true relationship between psoriasis and onychomycosis.

The common fungus isolated was Candida albicans (38.9%) followed by Trichophyton rubrum (33.3%), Trichophyton mentagrophytes (16.7%) and Epidermophyton floccosum (11.1%). Table 4 & figure 2. Zaias et al (1996) reported that the most common causes of onychomycosis are Trichophyton rubrum, Trichophyton mentagrophytes and Epidermophyton floccosum. Yeasts, particularly Candida albicans are mainly isolated from finger nails in chronic paronychia and onycholysis [4].

Probably, most fungi cannot infect a healthy nail organ, and only predisposing factors such as impaired blood supply, peripheral neuropathy, diabetes mellitus, damage from repeated minor trauma and limited immune defects make the nail susceptible to fungal infection [9]. The fungal infections in psoriatic patients might induce Kobnerization and delay or antagonize the effect of therapies for psoriasis of the nails and skin. Psoriasis has multiple aetiologies and T-cells play a critical role in the pathogenesis of this disease. It is possible that dermatophytes or yeasts may have a role in the causation or maintenance of psoriasis by acting as a superantigens[12].

Infection by dermatophytes (dermatophytosis) naturally stimulate the immune system as in those by other microorganisms to induce various immunological phenomena [18]. Cell-mediated immunity is critical to host defense against the fungal infection. Two functions critical to effective cell-mediated immunity, lymphoproliferation and cytokine release [13]. Interleukin-10 (IL-10) has major impact on immunoregulation since it inhibits proinflammatory cytokine formation and T cell mediated responses [11]. In this study all patients had regular systemic antipsoriatic therapy. Patients had increase level of IL- 10 (68.45 ± 53.009) than control (11.31 ± 1.947) P=0.0001 (table 6 &fig. 3). Patients with growth had increased level of IL-l0 (69.31 ±50.11) than patients with no growth (67.261 ±58.85) table 7 & fig. 4. Various studies mentioned that antipsoriatic therapies such as ultraviolet exposure or systemic immunosupressive drugs results in suppression of many cell mediated immune response and increase in T. helper type 2 cytokines such as IL- 10 [10,1].

Slight increase of interleukin- 10 level in patients with positive growth could play as a cofactor in enhancement of fungal infections by inhibiting T cell mediated responses. Similarly Kanani and Sussman (1999) reported increase incidence of cutaneous viral and fungal infections in atopic dermatitis patients with increased level of interleukin- 10. From this study it could be concluded that psoriatic patients had increase liability to onychomycosis which should be excluded by laboratory means prior to treatment of psoriatic nail with corticosteroid. Fungal infections may have a role in maintenance of psoriasis by acting as a superantigens. On the other hand, those patients had increase serum level of interleukin-10 which may predispose to infections by immunosupperssive effect.

REFERENCES

1. *Asadullah K; Sterry W; Stephanek K et al.* (1998): IL-10 is a key cytokine in psoriasis. Proof of principle by IL- 10 therapy; a new therapeutic approach. J. Clin. invest. 101:4, 783.
2. *Asadullah K; Sabat R; Wiese A et al.* (1999): Interleukin- 10 in cutaneous disorders, implications for its pathophysiological importance and therapeutic use. Arch. Dermatol. Res. 291:12, 628.
3. *Braverman M* (1972): Electron microscopic studies of the microcirculation in psoriasis. J. Invest. Dermatol. 59: 191.
4. *Evans E.G. VC.* (1998): Causative pathogens in onychomycosis and the possibility of treatment resistance: A review. Journ. Of the American Academy of Dermatol., 38 / 5: 3, S 32.
5. *Elewski BE* (1998): Onychomycosis; pathogenesis, diagnosis and management. Clin. Microbiol. Rev. 11: 3, 415.
6. *Feurman Alteras I and Aryelly J* (1976): The incidence of pathogenic fungi in psoriatic nails. Castellania 4: 195.
7. *Goldstein AO; Smith KM; Ives TJ et al.* (2000): Mycotic infections. Effective management of conditions involving the skin, hair and nails. Geriatrics 55:5, 40.
8. *Gupta AK; Wlynde C; Jam HC et al.* (1997): A higher prevalence of onychomycosis in psoriatics compared with non-psoriatics: a multicenter study. Brit. Jour. of Dermat. 136: 786.
9. *Haneke E* (1991): Fungal infections of the nail. Semin Dermatol. 10:1,41.
10. *Jones CD; Guckian M; El Ghorr AA et al.* (1996): Effects of phototherapy on the production of cytokines by peripheral mononuclear cells and systemic antibody responses in patients with psoriasis. Photodermatol. Photoimmunol Photomed. 12: 204.

11. *Kananii AS and Sussman GL* (1999): The role of infection in atopic dermatitis. J. Cutan. Med. Surge 312 :S2 -29 - S2.

12. *Leung DY; Travers TB; Giorno R* et al. (1995): Evidence for streptococcal superantigen-driven process in acute guttate psoriasis. J. Clin. Invest. 96: 2106.

13. *Levitz SM and North EA* (1997): Lymphoproliferation and cytokine profiles in human peripheral blood mononuclear cells stimulated by cryptococcus neoformans. J. Med. Vet. Mycol. 35:4, 229.

14. *Michel G; Gailis A; Jarzebska D.B. et al.* (1997): 1,25 (OH) 2-vitamin D3 and calcipotriol induce IL- 10 receptor gene expression in human epidermal cells. Inflamm. Res. 46:1, 32.

15. *Nickoloff BJ and Wrone. T* (1998): Superantigens autoantigens and pathogenic T cells in psoriasis. Investigat. Dermat. 110 : 4, 450.

16. *Peters BP; Weissman FG and Gill MA* (2000): Pathophysiology and treatment of psoriasis. Am. J. Health Syst. Pharm.57: 7, 645 - 659. Quiz 660.

17. *Romani L; Bistoni F and Puccetti P* (1997): Initiation of T-helper cell immunity to Candida albicans by IL-12; the role of neutrophils. Chem. Immunol. 68:110.

18. *Samman PD and Fenton DA* (1995): Psoriasis. In: Samman's the nails in Disease (Samman PD, Fenton Da eds.) Oxford: Butterworth / Heinemann 56.

19. *Talluri G; Marella VK; Shirazian D et al.* (1999): immune response in patients with persistant Candiduria and occult Candidemia. Jour. Urol. 162:4, 1361.

20. *Zaias N; Glick B and Rebell G* (1996): Diagnosis and treating onychomycosis. J. Fam. Pract. 42:5, 513.

دراسة عدوى الأظافر الفطرية و إنترلوكين ١٠ فى مرضى الصدفية

ميساء السيد - أمينة عبد العال - شيماء المنجى

إن مرضى الصدفية يعانون من أظافر غير طبيعية من الممكن أن يكون التشخيص الإكلينيكى صعبا لمعرفة إذا كان السبب يرجع الى الصدفية، عدوى الأظافر الفطرية أو إلى هذه الأسباب مجتمعة أو إلى أسباب أخرى.

إنترلوكين ١٠له مفعول مضاد للإلتهاب ومثبط للمناعة، وزيادة إنترلوكين ١٠من الممكن أن تؤدى إلى زيادة الإحتمال بالعدوى الفطرية الموجودة فى كثير من الأمراض الجلدية.

وهدف هذا البحث هودراسة دور المعمل فى تشخيص عدوى الأظافر الفطرية فى مرضى الصدفية والعلاقة بين العدوى الفطرية و مستوى إنترلوكين ١٠ فى المصل.

أخذت قصاصات أظافر من ٣١ مريض من مرضى الصدفية تحت العلاج ودرست بالميكروسكوب و بالزراعة على مستنبتات السابروید بالسيكلو هيكسيامید وبدون. و أخذت عينات من دم المرضى ومن ١٠أشخاص طبيعيين كمجموعة ضابطة وحدد مستوى إنترلوكين ١٠بالاليزا.

وجد أن ١٨ عينة (٥٨,١٪) إيجابية بالزرع للفطريات، وكان أكثر الفطريات شيوعا هو كانديدا ألبيكانس (٣٨,٩٪) يايه تريكوفيتون مينتاجروفيتس (١٦,٧٪) و إبيدير موفيتون فلوكوسم (١١,١٪).

بالنسبة للإنترلوكين ١٠فقد وجد أن معدلة أعلى بنسبة ملحوظة إحصائيا فى المرضى من المجموعة الضابطة (p=0.0001) وكان معدلة أعلى فى المرضى الذين لديهم عدوى فطرية (٥٠,١١+ ٦٩,٣١ بيكو جرام / مللى) عن المرضى الذين لم يكن لديهم عدوى فطرية(٥٨,٨٥ + ٦٧,٢٦ بيكو جرام / مللى).

من هذه الدراسة من الممكن الإستنتاج أن مرضى الصدفية يوجد لديهم إستعداد للعدوى الفطريةو التى يجب إستبعاد وجودها بالوسائل المعملية قبل العلاج للأظافر المصابة بالصدفية بالكورتيكو ستيروید .وان هولاء المرضي يوجد لديهم زيادة في معدل إنترلوكين ١٠ و التى من الممكن أن تزيد من تعرضهم للعدوى بالفطريات بتاثير ها المثبط للمناعة.