

THE CLINICAL SIGNIFICANCE OF CUTANEOUS REACTIONS TO TRICHOPHYTIN IN DERMATOPHYTOSIS

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Abstract. An intracutaneous test was performed in 114 patients with dermatophytosis verified by culture and in 32 control subjects, using purified trichophytin (according to the ethylene glycol method) and commercially available trichophytin. Immediate reactions occurred with both trichophytin antigens at a similar frequency in 12-14% of cases. Delayed reactions occurred significantly more often to the purified trichophytin in patients with dermatophytosis (36%) but in none of the controls. Immediate reactions to purified trichophytin occurred in 26% of the chronically infected patients. Delayed reactions were correlated to patients with tinea cruris (50% positive reactions), to infections with *Epidermophyton floccosum* (50%) and to patients not chronically infected. Thus, chronic dermatophytosis was characterized by immediate reactions to purified trichophytin and few delayed reactions. In contrast, patients not chronically infected were characterized by delayed reactions and few immediate reactions.

Key words: Dermatomycosis; Delayed hypersensitivity; Immediate hypersensitivity; Skin tests; Trichophytin

Since it was first developed in 1902, trichophytin as an intracutaneous antigen has been used in numerous investigations performed in patients with dermatophytosis (3, 13, 14). The results of these investigations have varied, and from a clinical point of view the conclusions have sometimes been conflicting. This is perhaps not surprising since the trichophytin antigens used have been of varied composition and have been produced by many different methods.

In 1960, Cruickshank and co-workers developed purified trichophytin and so provided a means to characterize and standardize the product and thereby facilitate the comparison of results of one clinical investigation with another (1, 2). However, many problems are still evident with regard to the

specificity and the significance of cutaneous reactions to trichophytin antigens.

The purpose of this study was to evaluate the results of intracutaneous tests in patients with dermatophytosis, comparing a commercial antigen with purified trichophytin, and to correlate cutaneous reactivity in this defined group of infected patients to the corresponding clinical conditions.

MATERIAL AND METHODS

The material consisted of 114 outpatients from the Department of Dermatology, Södersjukhuset, with clinical signs of dermatophytosis verified by culture. All patients were questioned about previous dermatophytosis and earlier diseases. Patients with immunological disturbances, immunosuppressive medication or atopic conditions were not included. Furthermore, 32 selected patients with various differing dermatological conditions, though without anamnestic or clinical signs of dermatophytosis, were tested as a control group.

Toe webs, inguinal areas, axillae and symptomatic areas were inspected, and cultures were taken when pathological conditions were observed. Specimens for culture were taken from all suspected areas and cultured on both Sabouraud's glucose agar and dermatophyte test medium (DTM) (17). Dermatophytes were identified in accordance with colony morphology and microscopic appearance using standard criteria (15).

All patients were tested on the volar aspect of the forearms with the following antigens simultaneously: 1) purified trichophytin processed at the Central Microbiological Laboratory, Stockholm, according to the ethylene glycol method (12). Each patient received intracutaneously 0.1 ml of the purified trichophytin diluted to 0.1 mg/ml. The antigen was stored in a frozen state, thereby avoiding the addition of preservatives. 2) a commercial trichophytin antigen obtained from Sächsisches Serumwerk KG, Dresden (Trichophyton Vaccine). Each patient received 0.1 ml of this antigen diluted 1:200 in saline (11). The tests were read after 20 min and 48 hours. The immediate reaction was considered positive when a distinct wheal of at least 11 mm diameter with surround-

Table I. Comparison of cutaneous reactivity to trichophytin antigens

	Patients tested	Pos. immediate reaction	Pos. delayed reaction	Pos. immediate and delayed
Purified trichophytin	114	14 (12 %)	41 (36 %)	2
Trichophyton Vaccine	114	16 (14 %)	26 (23 %)	2

NS

 $p < 0.01$

ing flare, or else a wheal with distinct pseudopodia appeared. The delayed reaction was considered positive when an induration and/or distinct erythema with a mean diameter of at least 4 mm appeared. Statistical analysis was carried out with Chi-square tests.

Furthermore, intracutaneous tests with tuberculin (PPD 2 TU, Statens Seruminstitut, Copenhagen) were performed in 20 of the patients with verified dermatophytosis.

RESULTS

Comparison of trichophytin antigens. One hundred and fourteen patients were tested intracutaneously with both purified and commercial trichophytin. As indicated in Table I purified trichophytin more often gave delayed reactions, viz. in 41 cases (36%), while the commercial antigen gave positive results in 26 cases (23%). This difference is statistically significant ($p < 0.01$). Immediate reactions occurred in 14 and 16 cases respectively. Both immediate and delayed reactions in the same patient occurred in 2 cases with both antigens. In the control group consisting of 32 patients no reactions whatsoever occurred to purified trichophytin. The commercial antigen gave positive delayed reactions in two cases. Immediate reactions were not registered.

Correlation between cutaneous reactivity and chronic infections. Those patients with mycotic infections of more than a year's duration with several relapses, or a total duration of more than a year, were considered chronically infected. As can be seen from Table II, the above-mentioned criteria were fulfilled in 43 patients. The results of the tests with purified trichophytin are shown in Table II. Immediate reactions were often seen in chronically infected patients (11 cases or 26%), while in the other group, reactions occurred in only 3 cases. This difference is statistically significant ($p < 0.01$). Delayed reactions occurred in the non-chronic group in 33 cases (48%) while 8 patients in the chronic group reacted positively. This difference is

also statistically significant ($p < 0.01$). Two patients with chronic mycosis reacted with both immediate and delayed reactions.

Correlation between cutaneous reactivity and the localization of the infection. As can be seen from Table III, two main diagnoses occurred, tinea pedis and tinea cruris. The two diagnostic groups consisted of 48 patients each, together accounting for about 90% of the patients tested. When comparing these two groups, it appears that delayed reactions occurred more often in tinea cruris than in tinea pedis, 24 cases (50%) and 11 (23%) respectively. This difference is statistically significant ($p < 0.02$). Thus half of the patients with tinea cruris reacted with a positive delayed reaction. No difference between the groups was registered with regard to the immediate reaction.

Correlation between cutaneous reactivity and the dermatophyte isolated. From Table IV it can be seen that *T. rubrum* is the most common dermatophyte isolated, followed by *E. floccosum* and *T. mentagrophytes*. This corresponds closely to the results of the dermatophytes isolated in the Department of Dermatology, Södersjukhuset, during this period. However, delayed reactions occur most often in infections with *E. floccosum* in 50% of cases, as compared with the *T. rubrum* group. The

Table II. Comparison of cutaneous reactivity to purified trichophytin in chronic and non-chronic patients

	Patients tested	Pos. immediate reaction	Pos. delayed reaction
Chronic dermatophytosis	43	11 (26 %)	8 (19 %)
Others (non-chronic)	69	3 (4 %)	33 (48 %)

 $p < 0.01$ $p < 0.01$

Table III. Comparison of cutaneous reactivity to purified trichophytin according to localization of infection.

	Pa- tients test- ed	Pos. immediate reaction	Pos. delayed reaction	
Tinea cruris	48	8 (17%)	24 (50%)	} NS } $p < 0.02$
Tinea pedis	48	4 (8%)	11 (23%)	
Others	18	2	3	

difference is statistically significant ($p < 0.01$). However, *T. mentagrophytes* gave 8 positive reactions (66%), but the patients were too few to allow statistic analysis with the Chi-square test. No statistical difference between the groups was registered concerning the immediate reactions.

Reactivity to tuberculin. Of the patients tested 7 were chronically infected and 13 had non-chronic infections. No differences were registered between these two groups in delayed hypersensitivity.

DISCUSSION

Purified trichophytin has been considered a specific antigen indicating present or past dermatophytosis, although the sensitivity of the antigen varies (4, 7, 18). In this study, there is no significant difference in reactivity with respect to immediate reactions, when comparing the two antigens used. However, the delayed reactions showed, a statistically significant difference: 36% reacted to purified trichophytin and 23% to commercial trichophytin. It would thus seem, that the purified antigen is more specific. Moreover, no false-positive reactions to purified trichophytin occurred in the control group. However, only 36% of the patients with verified dermatophytosis responded to purified trichophytin with a delayed reaction. Recent studies with crude and purified trichophytin show a quite similar frequency, as in this study—about 40–60% positive reactions with no or few reactions in non-infected subjects (4, 5, 7). Thus it seems that purified trichophytin is specific in indicating dermatophytosis, but its sensitivity is rather low.

The immediate reaction. For many years (6) the immediate reaction has been considered to be as-

sociated with *T. rubrum* infections, but in recent investigations (7, 9, 16) it has been correlated to chronic dermatophytosis. The nature of this reaction is not fully understood but it is thought to be a reagin-mediated reaction.

The frequency of the immediate reaction is rather low, 12% in this investigation, but corresponds well to the results found in other studies with purified trichophytin (4, 7, 18). However, from the results of this study it seems that the immediate reactions are correlated to chronic infections (Table II). Approximately 80% of the immediate reactions are seen in the chronic patients, while only 3 patients of 69 in the non-chronic group reacted. Considering reactions to *T. rubrum*, immediate reactions occurred frequently but the material does not permit statistical analysis. However, approximately 80% of the immediate reactions occurred in infections with *T. rubrum*. Thus, in this study, a correlation could be established only between the immediate reaction and chronic mycosis.

The delayed reaction. Delayed cutaneous hypersensitivity to trichophytin is considered to be a type IV reaction indicating present or past dermatophytosis and also present in "id" reactions. Furthermore, there is reason to believe that the delayed reaction reflects a certain state of immunity to dermatophytosis, probably partial and of limited duration (7, 8, 10, 13). Patients with chronic dermatophytosis seldom react to purified trichophytin (7). In this study, delayed cutaneous reactions correlate to non-chronic patients (Table II). Only 8 of the chronic patients reacted, and 5 of these were infected with *T. mentagrophytes* which is known to elicit delayed reactions frequently (4).

The dermatophytes differ in their ability to induce delayed reactions. It is well known that certain

Table IV. Comparison of cutaneous reactivity to purified trichophytin according to the dermatophyte isolated

	Pa- tients test- ed	Pos. immediate reaction	Pos. delayed reaction	
<i>T. rubrum</i>	60	11 (18%)	11 (18%)	} NS } $p < 0.01$
<i>E. floccosum</i>	38	2 (5%)	19 (50%)	
<i>T. mentagrophyte</i>	12	1	8 (66%)	
Others	4	0	3	

dermatophytes, especially zoophile strains with inflammatory properties, often give delayed reactions (3). In contrast, *T. rubrum* which often gives a low-grade inflammation and a superficial infection seldom demonstrates delayed reactions (4). *E. floccosum* has also been considered as a "non-reactant" in this respect, similar to *T. rubrum* (5). In this study, infections with *E. floccosum* are closely correlated to delayed reactions, i.e. giving positive reactions in 51% of the cases. Moreover, this frequency is comparable to that of *T. mentagrophytes*, 51 and 66% respectively.

Thus it seems that *E. floccosum* might be a dermatophyte that gives more deep-seated lesions than earlier thought. In this respect the information concerning *E. floccosum* seems to be sparse, and to my knowledge only a few patients with *E. floccosum* infections have been tested with purified trichophytin. Nevertheless, in this study delayed reactions are correlated to infections with *E. floccosum*. *T. rubrum* gave only positive delayed reactions in 11 cases (18%) which is consistent with earlier findings (4, 18).

The localization of infection could also be of importance in reactivity. It is well known that *tinea pedis* seldom reacts with delayed hypersensitivity, but whether this is due to the high frequency of chronic reactions, infections with *T. rubrum*, or to local aberrations of the foot as an immunological reactor is not known. *Tinea cruris* has also been considered seldom to react but in this study infections in the groins often produced delayed reactions. Delayed reactions occurred in 50% of the cases, as against 23% in *tinea pedis*, and this is a statistically significant difference.

It is possible that different localizations of the infections predispose to sensitization in different ways. It is also possible that the close and intimate contact of the dermatophyte in this intertriginous area with its thin epidermis, facilitates the exposure of the antigen to the immune system manifesting as a cutaneous reaction and possibly indicating certain immunity. Furthermore, only 7 of the 48 patients with *tinea cruris* were chronically infected.

From a clinical point of view it might be justified to draw the following conclusions from this investigation:

1. Purified trichophytin is a more reliable antigen for the indicating of dermatophytosis than is commercially obtained trichophytin.

2. Chronic dermatophytosis is characterized

by immediate reactions to trichophytin and few delayed reactions.

3. Delayed reactions to purified trichophytin might indicate a certain immunity to dermatophytosis, as non-chronic patients are characterized by delayed reactions and few immediate reactions.

4. Infections with *E. floccosum* are correlated to delayed reactions, thus conflicting with previous knowledge. *E. floccosum* might possibly be considered an inflammatory dermatophyte in this respect.

5. *Tinea cruris* is correlated to delayed reactions, thus indicating the importance of the localization of infections.

Further studies with purified trichophytin are needed before the above conclusions can be fully accepted. It is interesting to observe, however, the relative consistency in the observations concerning delayed reactions to purified trichophytin. It seems as if the delayed reaction could serve as an indicator of the immunological status of an individual, and thereby possibly predict the likely course of a dermatophyte infection and the risk of chronicity. It could even serve as a guide to the intensity of the therapy used.

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