

# Dermatophytes and Keratin in Patients with Hereditary Palmoplantar Keratoderma

## A Mycological Study

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**Fourteen patients with hereditary palmoplantar keratoderma of the Unna Thost variety were included in the study. Dermatophytosis was found in 7 of the 14 patients. Six were affected with *T. rubrum* and one with *T. mentagrophytes*. The growth pattern of dermatophytes in keratin from the patients did not differ from that of normal control individuals. Keratin from patients with hereditary palmoplantar keratoderma was sterilized with ethylene gas and placed in the center of culture plates, previously broad inoculated with control dermatophytes or dermatophytes isolated from patients. An inhibition zone around the keratin was found in 42.9% of the control dermatophytes and in 83.4% of the patient cultures. The inhibition zone was only seen in cultures with *T. rubrum* and not in those with *T. mentagrophytes*. No significant difference in minimal inhibitory concentration values against ketoconazole between control dermatophytes and dermatophytes from patients was demonstrated.**

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The frequency of hereditary palmoplantar keratoderma (HPPK) of the Unna Thost variety in the northernmost county of Sweden (Norrbotten) has been shown to be 0.55% (1). It has also been documented that the prevalence of dermatophytosis was 36.7% with reference to conventional culture and to direct microscopic examination 41.7% (2, 3).

Keratin from palms and soles apparently possesses a pronounced affinity to dermatophytes. However, no differences between the amino acid composition of keratin from patients with HPPK and that of normal keratin were found (4). The pH of skin of soles did not differ from that of normal individuals and, therefore, the affinity of dermatophytes to the horny layer could not be interpreted as an increased activation of the alkaline dermatophyte keratinases (5).

As no biochemical differences were demonstrated, it was considered of interest to study the effect of keratin from patients with HPPK and healthy control individuals on both dermatophytes isolated from patients and control dermatophytes. The sensitivity of dermatophytes isolated from patients with HPPK to ketoconazole was also compared with the sensitivity of dermatophytes from normal individuals.

## MATERIAL AND METHODS

### Characteristics of patients

Fourteen patients (6 women and 8 men) with a mean age of 49 years (range 22–79 years) suffering from HPPK of the Unna Thost variety

were included in the study. Patients had not been treated with antimicrobics or topically applied salicylic acid for at least 2 months before they entered the study. Five healthy subjects (2 women and 3 men) with a mean age of 43 years (range 26–54 years) who had never suffered from dermatophytosis, served as controls.

### Collection of keratin

Keratin was collected by curettage from the right and left sole of patients and control individuals.

### Collection of fungi

Skin scales for fungal culture were collected with a curette and inoculated on Sabouraud's glucose agar without cycloheximide. Dermatophytes from the initial cultures were transferred to Sabouraud's glucose agar with cycloheximide as additive and inoculated at 32°C for up to 3 weeks. Laboratory dermatophyte cultures from healthy individuals (one *T. rubrum* and one *T. mentagrophytes*) originated from Mycological Laboratory, Department of Dermatology, University Hospital, Gothenburg, Sweden, served as control cultures.

### Experimental procedure

Curettaged keratin from patients and controls was sterilized with ethylene gas (6). Ethylene gas has an antimicrobial capacity and does not interfere with or denature proteins. Dermatophytes isolated from patients with HPPK were broad-inoculated on Sabouraud's glucose agar. Sterilized keratin from soles of the corresponding patients were then placed in the centre of the plate. Likewise, control dermatophytes were inoculated on plates together with sterilized keratin from the soles of all 14 patients with HPPK included in the study. Controls were also dermatophytes from patients inoculated with sterilized pooled keratin from healthy individuals. Cultures were incubated at 32°C and macroscopically and microscopically read after 1 week. Microscopic examination was performed using ultra thin Scotch tape 830 3M (Los Angeles, California, USA) stained with lactophenol cotton blue. Two preparations were made from each colony; one with a slight pressure onto the fluffy mycelium and one with a more vigorous pressure onto the centre in order to attach keratin to the tape.

### Estimation of minimal inhibitory concentration (MIC)

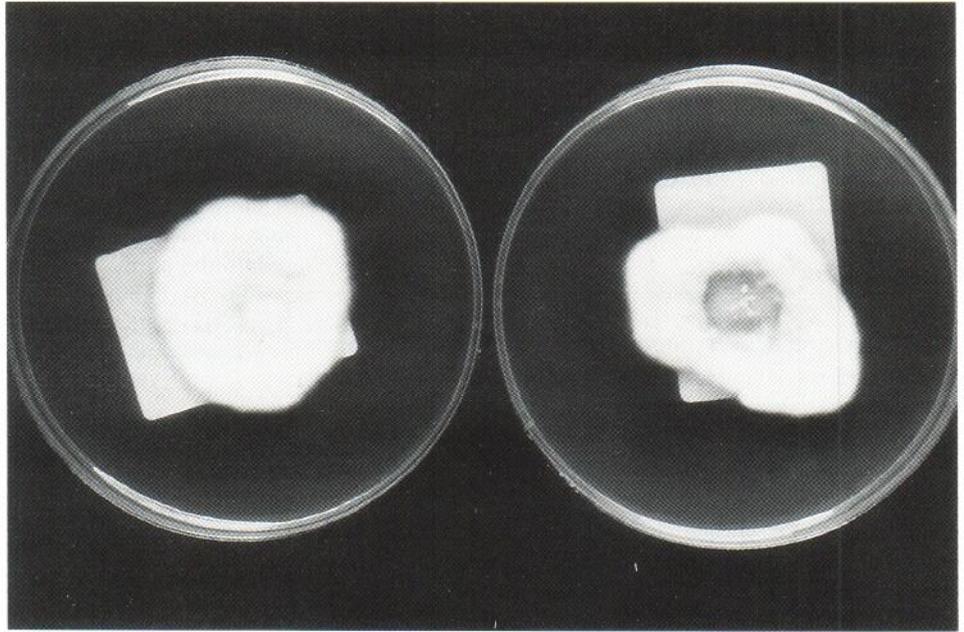
Estimation of MIC of ketoconazole was included to see if there was any difference between the sensitivity of dermatophytes from patients and to control dermatophytes.

The fungi investigated included 6 isolates of *T. rubrum* and one isolate of *T. mentagrophytes* from soles of patients with HPPK. *T. rubrum* and *T. mentagrophytes* obtained from the Mycological Laboratory, Department of Dermatology, University Hospital, Gothenburg, Sweden, served as controls. The experiment was repeated twice.

### Test substance

Ketoconazole was obtained from Janssen Pharmaceuticals, Gothenburg, Sweden.

Fig. 1. Inhibition zone produced by sterilized keratin from a patient with hereditary palmoplantar keratoderma and *T. rubrum* infection compared to a negative control culture.



#### Test medium

The test medium was DST (Oxoid, UK).

#### Experimental procedure

Ketoconazole was dissolved in DMF (dimethylformamid) in a concentration of 1000 µg/ml. Specimens from this stock solution were added to DST medium to give concentrations of 100, 50, 12.5, 6.25, 3.1, 1.6, 0.8, 0.2, 0.1 and 0.05 µg/ml. Control plates were made with DMF and sterile water. Dermatophytes were harvested after 1 week of growth. The mycelium was dispersed in PBS (phosphate-buffered saline, pH 7.2) and crushed in a sterile mortar. The suspensions were then filtered through filter gauze and adjusted to an optical density of 65% transmission at a wavelength of 550 nm (Beckman Model 24. Spectrophotometer). From these solutions 0.1 ml was inoculated on DST medium. Cultures were incubated at 32°C and read after 4 days' growth. MIC was assessed as the concentration of ketoconazole, where a total inhibition of growth was seen (7).

#### Statistics

For statistical calculations the Wilcoxon rank sum test was used.

## RESULTS

Clinical signs of dermatophytosis on soles of patients with HPPK were found in 5 out of 15 patients and dermatophytes were isolated from 7 out of 14. Unilateral isolation of dermatophytes

was performed from 3 and bilateral isolation from 4 patients. The results of cultures from patients are shown in Table I.

When dermatophytes were reinoculated on Sabouraud's glucose agar together with the homologous keratin, an inhibition zone was demonstrated in the centre of 5 out of 6 cultures with *T. rubrum* from patients with HPPK (Table II). No inhibition zone was seen with keratin from control individuals. Control cultures of *T. rubrum* and *T. mentagrophytes*, incubated together with keratin from 14 patients with HPPK, showed an inhibition zone in 6 cultures (Table III).

Microscopic examination of tape specimens stained with lacto phenol cotton blue revealed no difference in growth pattern of dermatophytes between control keratin and keratin from patients with HPPK. In samples without growth inhib-

Table I. Fungi isolated from the right, the left and both soles of 14 patients with hereditary palmoplantar keratoderma

	Both soles	Right sole	Left sole	Total
<i>T. rubrum</i>	3	3	0	6
<i>T. mentagrophytes</i>	1	0	0	1
<i>E. floccosum</i>	0	0	0	0
<i>Candida albicans</i>				
+	0	0	1	1
++	0	0	0	0
+++	2	0	0	2
Contaminants	1	0	2	3
Negative	4	0	0	4

Table II. Dermatophyte cultures from patients with hereditary palmoplantar keratoderma inoculated with their homologous scraped keratin and control dermatophytes inoculated with pooled control keratin

Pat. No.	Fungus	Inhibition zone		
		0-5 mm	5-10 mm	>10 mm
1	<i>T. rubrum</i>		+	
2	<i>T. rubrum</i>			+
	<i>T. rubrum</i>			+
3	<i>T. rubrum</i>			+
5	<i>T. rubrum</i>		+	
	<i>T. rubrum</i>		+	
7	<i>T. mentagrophytes</i>			
	<i>T. mentagrophytes</i>			
11	<i>T. rubrum</i>		+	
14	<i>T. rubrum</i>	+		
	<i>T. rubrum</i>		+	
Control	<i>T. rubrum</i>			
	<i>T. mentagrophytes</i>			

Table III. *T. rubrum* and *T. mentagrophytes* cultures, from the Mycological Laboratory, incubated together with keratin from 14 patients with hereditary palmoplantar keratoderma

	Inhibition zone (patients)		
	0-5 mm	5-10 mm	>10 mm
<i>T. rubrum</i>	2	0	4
<i>T. mentagrophytes</i>	0	0	0

ition, hyphae on the keratin scale area were thick with a high density and an abundant presence of chlamydospores and microconidies. Macroconidies were, however, not present. At the transition zone to the agar surface hyphae, chlamydospores and microconidies occurred scantily with no difference between control dermatophyte cultures and cultures from patients with HPPK. Inside the inhibition zone hyphae were broken up into arthrospores mixed with radiating thin hyphae without microconidies or chlamydospores. Gradually all fungal elements completely disappeared. Examination of the fluffy mycelium showed thin hyphae and no other fungal structures.

The MIC of ketoconazole against the 6 *T. rubrum* and one *T. mentagrophytes* from patients with HPPK was lower (0.767, SD  $\pm$  0.65) than the MIC against *T. rubrum* and *T. mentagrophytes* control isolates (2.56, SD  $\pm$  2.15). However, no statistically significant difference was found ( $p = 0.1025$ ).

#### DISCUSSION

It is well known that patients with HPPK more often are infected with dermatophytes in the hyperkeratosis of palms and soles. It has been postulated that this affinity to dermatophyte infections is due to a very thick layer of keratin (8). If this is the correct or only explanation why dermatophytosis more often occurs in these patients, this subject has not yet been studied.

It has been published that *T. mentagrophytes* is a weak homologous and heterologous antibody inducer compared to *T. rubrum* and *E. floccosum* and that patients with HPPK infected with *T. mentagrophytes* do not produce higher levels of IgG antibodies against *T. rubrum*, *T. mentagrophytes* or *E. floccosum* than healthy control individuals (9). Activation of the immune system may start an inflammatory reaction and may be one explanation for the vesicles and redness at the transition zone between hyperkeratosis and normal skin, often seen in patients with HPPK infected with *T. rubrum*.

Treatment of keratin with ethylene gas has an antimicrobial effect without denaturing proteins. The inhibition zone was only seen with keratin from patients with HPPK and it was induced by *T. rubrum* isolated both from patients and control

strains. This indicates that it must be a substance present only in keratin from patients with HPPK. Further studies have to be made to characterize this substance.

It has recently been reported that antimicrobial immunoglobulins may reach the stratum corneum through sweat and sebum (10, 11). Most patients with HPPK suffer from hyperhidrosis and owing to the hydrophilic character of the hyperkeratosis, immunoglobulins would immediately be absorbed into the horny layer. Therefore, immunoglobulins could possibly be found in the outermost layers of stratum corneum reacting with approaching antigenic invaders. An immunological reaction might explain the inhibition zone found in cultures of *T. rubrum*. Generally it is more difficult to treat dermatophyte infections in patients with HPPK than in those without (12). However, significant differences in MICs of ketoconazole against control dermatophytes or dermatophytes isolated from patients could not be demonstrated. Therefore, the reason for treatment failures is probably due to factors in the patient.

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