Yeast Infections as a Cause of Nail Disease In the Western Province of Saudi Arabia

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Onychomycosis is a worldwide problem especially in tropical areas. It is caused by three groups of fungi; dermatophytes, other molds and yeasts. A high prevalence of Candida infections of the nails has been reported in many countries.

Aim: This study was conducted to find out the incidence of fungal infection in suspected cases of onychomycosis and to identify the causative fungi in such cases.

Method: A 10 months study was conducted randomly on 54 patients (32 females and 22 males) ranging in age between 13 and 84 years old with suspected fingernail or toenail fungal infection. Nail clipping or scrapping were collected, examined with potassium hydroxide preparation (KOH) for direct microscopy and cultured for fungi.

Results: Of the 54 cases, fungi were isolated in 36 of the collected sample by KOH and in 29 cases by culture. Most females (18) presented with fingernail fungal infection while most males (13) presented with toenail fungal infection. Cultures showed that all the isolates were Candida species; *C. albicans (72.4%), C. parap-silosis (20.7%),* and *C. guilliermondii* (6.9%).

Conclusion: These data revealed that candidal infection are responsible for nail affection in suspected cases of onychomycosis and by cultural identification. *C. albicans* was found to be responsible for higher percentage followed by *C. parapsilosis* then *C guilliermondii*.

Keywords: Onychomycosis, yeast, Candida, *C. albicans.*

Onychomycosis is a worldwide problem[1,2]. It constitutes 15-22% of all nail diseases[3], about 2-5% in adult population in Europe[4,5]. It is very common in the Dermatology Clinics of Countries of Middle East. Although the infection is very common, most people with this problem do not seek medical advice[6].

Mycotic infection of the nails can be caused by three groups of fungi; (1) Dermatophytes especially in temperate zones, (2) Other molds depending on geographic region, both toenails and fingernails can be affected by primary invasion of the healthy nail plate usually in association with adjacent areas of skin, and (3) Yeasts[7,8,9]. Although the aetiological agents of onychomycosis are well established in many countries, recently onychomycosis caused by uncommon fungi has been reported[6].

A high prevalence of Candida infections of the nails has been reported in Saudi Arabia[10], India[11] and Thailand[12].

The infection rates and types of fungi involved in onychomycosis vary with conditions such as age, sex, occupation, hygiene, footwear, environmental and climate factors[13,14].

In Saudi Arabia, there are many promoting factors including social and environmental factors but the extent is not exactly known. The **aim** of this study was to investigate patients with suspected fungal nail infections attending the Outpatient Dermatology Clinic in Fakeeh Hospital to find out the incidence of fungal infection in cases with suspected onychomycosis and to identify the different species of fungi isolated from such cases.

PATIENTS AND METHODS:

A- PATIENTS:

Fifty-four patients with clinically suspected onychomycosis attending the outpatient Dermatology Clinic in Fakeeh Hospital, Jeddah, western province of Saudi Arabia were randomly subjected to this study (32 females and 22 males). The study was conducted along a ten months duration (from October 2003 to July 2004). The ages of the included cases ranged between 13 and 84 years old and their disease duration ranged between one month and seven years. The patients were included irrespective of their occupation, systemic diseases as Diabetes Mellitus, peripheral vascular diseases, immunodeficiency or endocrinopathy. All patients had no history of nail trauma, drug intake or exposure to chemical materials.

Complete physical and dermatological examination were done to exclude any diseases especially psoriasis, eczema and lichen planus.

The criteria for suspecting onychomycosis include any of the following: nail discolouration, (whatever it is; yellowish, whitish or other) loss of luster, distal onycholysis, subungual hyperkeratosis or debris, nail ridging, irregular nail surface, weakness, separation of the nail plate from the nail bed (whether distal or lateral), thickening of nail plate, striations (longitudinal or transverse). The age, sex and clinical data of the included patients are shown in Table (I).

B- METHODS:

1- Collection of Specimens:

Suspected nail or the most affected nail was cleaned with 70% V/V ethanol. Nail pieces were collected either by taking snippings of infected part of the nail using arterial scissors to get full thickness of nail sample or scrapped using arterial scalpel on a clean piece of paper (about 5 cm square). If any debris underneath the nail, these debris were carefully sampled with sterile needle. Then the paper was folded to form a flat packet. It was closed with paper clip. Then the specimen was labeled with the patient's name, number and date. The specimen were delivered to the hospital laboratory[15].

2- Direct Microscopy:

The small nail pieces, more than 2 mm across, were deposited onto a clear microscopic slide, a cover slip is placed over the collected debris and a drop of clearing solution (10% potassium hydroxide) is carefully placed on the edge of cover slip and gentle heating. The fungal elements resist the clearing solution (KOH) because of their chitinous cell wall.

The specimen was examined microscopically using the 10X and 40X objectives. In a positive preparation fungi appeared typical round to oval budding cells[15].

3- Culture Procedures:

The clinical specimens (nail) were subcultured after cutting into pieces as small as possible. For each sample, two slants of Sabouraud's dextrose agar with and without 0.5 µg/ml of cycloheximide and 16 µg/ml of chloramphenicol to inhibit the growth of contaminating molds and bacteria, and one slant of dermatophyte test medium for primary recovery of dermatophytes DTM (as screening medium).

Culture were incubated at room temperature (25-30°C) and examined periodically for growth of yeast and dermatophytes. Negative culture are discarded after 30 days[16].

4- Identification and Speciation of the Dermatophyte and Yeasts:

This requires careful observation of gross colonial morphology and microscopic examination of properly prepared samples. All colonies of yeasts were identified by the Yeasts Biochemical Card (bioMérieux Vitek 2 Hazelwood, M0). It is a 30-well disposable plastic card that contains 26 conventional biochemical tests and 4 negative controls. The biochemical card was used with the automated AutoMiocrobic System. Correctly identification were reported after 24 hours of incubation. It was performed according to manufactures[17].

RESULTS:

Fifty-four cases had been subjected for this study; 32 females (59.3%) and 22 males (40.7%). Their age ranged between 13 and 84 years (mean 35.1 ± 12.2). The duration of their nail complaints ranged between one and 84 months (mean 19.5 ± 15.3). Their occupations were variable (20 were house wives

(37.7%), 15 were professionals (28.3%), 7 were manual workers (13.2%), 7 were students (13.2%) and the remaining 4 cases were not working (7.6%).

Of the 54 clinically suspected cases of onychomycosis included in this study, direct microscopy was positive in 36 of cases (66.7 %) while the remaining 18 cases (33.3 %) showed negative results. Cultures were positive in 29 patients (53.7 %), while 25 patients (46.3%) gave no growth, Table (2).

In the 36 cases showing positive direct microscopy cultures yielded positive results in 25 cases (69.4%) while 11 cases (30.6%) showed negative cultures. In the 18 cases, showing negative direct microscopy, cultures were positive in 4 cases (22.2%) and the remaining 14 cases were negative (77.8%), Table (3).

Candida species were the pathogen to be isolated from the cultures. Identification of the isolates showed that ***Candida albicans*** constituted 72.4% of the 29 positive cultures (21 cases), *C. parapsilosis* constituted 20.7% of the positive cultures (6 cases) while *C. guilliermondii* constituted 6.9% of the positive cultures (2 cases),Table (4).

In relation to the total number of cases with suspected fungal nail infection (54 cases) candida albicans was isolated in 38.9% of cases, C. *parapsilosis* was isolated in 11.1% of cases, while C. *guilliermondii* was isolated in 3.7% of cases, Table (5)

Fingernails were affected in 21 cases (38.9%), toenail in 22 cases (40.7%) while both were involved in 11 cases (20.3%). Involvement of finger nails in females was found in 18 cases (56.2%), while toenail involvement in males was found in 13 cases (59%). Table (6).

Rightside (whether hand or foot) was involved in 18 cases (33.3%), left hand in one case (1.9%) while both sides were involved in 35 cases (64.8%). Middle and index fingers were the commonly involved fingers in the hands while the big toe was the commonly affected toe in the feet.

Discussion:

Onychomycosis is clinically defined as infection of nail caused by any fungus including dermatophytes, non-dermatophytes and yeast. Candida onychomycosis is seen in patients with chronic mucocutaneous candidiasis. Both toenails and finger nails are affected. The surface of the nail becomes opaque, rough, and furrowed. It is usually discolored and may be brownish-yellow in color [18].

Candida species particularly *C. albicans* can also cause distal or lateral onycholysis, paronychyia and total nail dystrophy in rare cases of chronic mucocutaneous candidosis. Although this yeast is a member of the normal flora of the alimentary tract, it is not a normal inhabitant of the intact skin, and when it occurs in nail disease, it is assumed to be of pathogenic significance[19].

Fungal nail infections are almost exclusively an adult-malady. Children can be affected during household epidemics, but the faster nail growth in children appears to make infection more difficult. Mold infections usually affect the elderly whose underlying nail diseases allow room for these secondary invaders[20].

Using reliable criteria, our survey in 54 of suspected cases of onychomycosis, cultural isolates were positive for mycotic infection in 53.7% of suspected cases of onychomycosis (29 cases). These isolates have shown that *Candida albicans* represented 72.4 % of the positive cultures, *Candida parapsilosis* represented 20.7 % of the positive cultures and *Candida guilliermondii* represented 6.9 % of the positive cultures.

This observation corresponds with observation reported by **Al-Sogar et al. in 1991** who reported that Candida species seem to be too frequent in Eastern province of Saudi Arabia due to social and climatic factors[10] and by **Midgley et al. in 1991**, who reported 38% and 35% of *C. albicans* and *C. parapsilosis* respectively from isolated yeasts of nail infections[19].

Also, our results are consistent with the results of **Khafagi et al., 1998** who found that, molds and yeasts represented 50%-34.4% of isolates respectively while dermatophytes represented 15.6% of the isolates[27]. High prevalence of yeast infections were also reported by **Banerjee et al.,1989** in India[11], **Clayton ,1992** in United States[7], and **Nsanze et al., 1995** in United Arab Emirates[6].

On the other hand, **Summerbell et al.,1989** found that 91% of fungal infections were caused by dermatophytes, 6% by *Candida species*, 3% by non-dermatophyte moulds[25]. On the other hand,

Elewski and Hay 1996 reported that 90% of fungal nail infection were caused by dermatophytes, 7% by *Candida* and 3% by non-dermatophyte moulds**[26]**. The cause of such diversity may be explained by the fact that such studies were conducted in other countries that are different from Saudi Arabia in climate, environmental and social factors.
Mycotic infection was more common in females (62.1 %) than in males (37.9 %) of the positive cases with fungal infection. Although toenail fungal infection (40.7 %) was more common than fingernail infection (38.9 %); higher incidence of fingernail affection had been found in females (56.2%) , while toenail infections were more observed in males (59 %).
These findings are consistent with the observation of **Nsanc et al. in 1995,** who attributed the higher incidence of fingernail infections in females to the fact that females commonly do more domestic hand-washing, laundry, handling foodstuffs and often come in contact with raw meat than males**[6]** and reflects the greater burden of wet work done by females [21].
While high incidence of toenail infection in males could be attributed to the footwear which is mainly in the form of open slippers that predispose males to toenail trauma that is followed by secondary mycotic infections including yeast. Also, males practice activities in the desert without protective shoes, in addition, bathing facilities in gymnasia after sport; all these factors are incriminated in toenail infections in males**[24]**.
Onychomycosis in more frequently observed in the right hand than left hand especially middle and index fingernails. This could be explained by the fact that most of patients were right handed. While in the feet, it is more frequent in the big toenail. However, fungal infection by yeast usually involves several nails and sometimes all may be affected.
In the present study, from the 54 suspected cases of onychomycosis 66.7 % showed positive microscopy for fungal element while 33.3 % showed negative results. On the other hand, 53.7 % gave positive culture for fungal element while 46.3% gave negative results.
These results are similar to the results reported by **Midgley et al. in 1994[19]**, who obtained positive results in 62% of 168 patients with onychomycosis, **Khafagy et al. in 1998,** who obtained positive microscopy for fungal element in 87% of cases and 13% showed negative results, while 75.4% gave positive culture for fungi and 24.6% gave no growth. It has been hypothesized that diagnostic accuracy of routine methods for mycological examination may be as low as 50-70% and other laboratory investigations such as histological examination and immunochemistry can provide a more accurate diagnosis. So good differential diagnosis, proper collection of nail specimen, correct media and suitable temperature for incubating the inoculated media play a great role in the mycological results for onychomycosis**[27]**.
In the present study from 36 positive KOH direct microscopy, 11 cases (30.6%) yielded negative cultures. This could be explained by that those patients may receive incomplete, interrupted, improper courses of antifungals. From the 18 negative KOH direct microscopy, 4 cases (22.2%) yielded positive results. This could be attributed to insufficient sample collected for examination. Accordingly, sensiti-vity of KOH direct microscopy is 86.2%, while specificity is 56%.
To explain the cause of such diversity, Zaias in 1969 stated that , microscopy may frequently be negative in nails that clinically appear to be infected, and nails that are positive by microscopic examination often yield negative culture. A reason for this discrepancy is that fungi seen on KOH examination may not be a viable and hence do not grow as expected. Since the most commonly sampled area of the nail is the distal tip with its associated subungual debris, we must re-evaluate whether better result could be obtained by devising better sampling methods**[22]**.
No evidence here to suggest a mixed infection on obtaining the results whether on direct microscopy or culture. That is because on direct microscopy, the samples were taken carefully then and transferred on sterile dishes. And because culture were kept on media containing cycloheximide.

CONCLUSION:

In any nail change, such as discolouration, abnormalities in nail surface as ridging, loss of lusture, fragility, separation of nail plate from the nail bed, onycholysis, you have to consider onychomycosis until proved otherwise.

Concerning the causative fungi of onychomycosis, it was found that yeasts shared dermatophytes in responsibility of nail infections. The relative percentage of cases according to causative fungi depends on geographic location.

In the present study, yeasts were isolated in the following order of incidence, *C. albicans* > *C. parapsilosis* > *C. guilliermondii*.

Incriminating yeast as a primary cause of onychomycosis is not a simple judgement. So, it is therefore essential in cases where yeasts are implicated as primary pathogen to correlate the results of direct microscopy with those of the isolated culture.

Although the numbers in this study were small, they confirm earlier reports of prevalence of yeast infection in S.A.

Table (1): **Clinical data of the included patients**

Item	Number	Percentage
Age		
Minimum	13 Years	
Maximum	84 Years	
Man ± SD	35.1 ± 12.2	
Sex		
Female	32	59.3%
Male	22	40.7%
Duration		
Minimum	One month	
Maximum	7 Years	
Man ± SD	19.5 ± 15.3	
Occupation		
Housewife	20	37.7%
Professional	15	28.3%
Manual Workers	7	13.2%
Students	7	13.2%
Non works	4	7.6%

Table (2): **Results of Direct Microscopy and culture**

		NO.	%
KOH Direct Microscopy	+ VE	36	66.7
	- VE	18	33.3
Culture	+VE	29	53.7
	- VE	25	46.3%

Table (3) **Interpretation of KOH and Culture Results.**

P. Value

KOH	CULTURE		TOTAL	
	+ VE	- VE		
+ VE	25 (69.4%)	11 (30.6%)	36	0.001 (significant)
- VE	4 (22.2%)	14 (77.8%)	18	
TOTAL	29	25	54	

Table (4): **Results of mycological culture of the positive cases**

Positive (29) Cultures	NO	%
C. albicans	21	72.4
C. Parapsilosis	6	20.7
C. guilli-crmondii	2	6.9

Table (5) : **Interpretation of KOH and mycological identification of the isolates**

KOH	CULTURE					P Value
	+ VE				- VE	
	TOTAL	C.A.	C.P.	C.G		
+ VE (36)	25	19	5	1	11	0.007 (Significant)
- VE (18)	4	2	1	1	14	
TOTAL	29 53.7%	21 38.9%	6 11.1%	2 3.7%	25 46.3%	

Table (6): **Finger or Toe affection of females and males**

SEX	FINGER NAIL	TOE NAIL	BOTH	P VALUE
FEMALE	18 56.2%	9 28.1%	5 15.6%	0.007 (Significant)
MALE	3 13.6%	13 59%	6 27.3%	

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