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Research Article

ONYCHOMYCOSIS, ONYCHOLYSIS *IN VITRO* SUSCEPTIBILITY OF ISOLATES

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ABSTRACT

Introduction: Onychomycosis is the term used to describe the fungal infection of nail units and onycholysis is described as painless separation of nail plate from its nail bed. It is reported to have an incidence of 0.5 - 5% population worldwide.

Materials and methods: 100 patients clinically suspected to have onychomycosis were taken up for this study. Nail scrapings and clippings were obtained from deeper part of discoloured or dystrophic parts of nail. All samples were subjected to direct microscopy (40% KOH) and for culture, all samples were inoculated onto three sets of Sabouraud's dextrose agar with cycloheximide and Sabouraud's dextrose agar with cycloheximide and chloramphenicol and incubated at 25˚C and 37˚C and observed for growth for six weeks.

Results: Out of 100 patients examined, 42 were males and 58 females.Out of these 100 patients 49 cases yielded growth on culture (culture positive) and 46 cases were positive in direct examination (KOH positive). Out of 100 patients suspected of onychomycosis, 27 cases were found to be *Candida albicans*, 4 cases infected with dermatophytes , 12 cases infected with non-dermtophytic moulds , 3 cases of *Trichosporon spp* and 2 *Geotrichium* cases .

Conclusion: In conclusion, our study shows that, fingernails in females were more infected with Candida species, microtrauma being most common cause and often associated with paronychia and nondermatophytes were predominant in toenail infections associated with occlusive footwear.

Keywords: Onychomycosis, Candida, Non –Dermatophytic Moulds.

INTRODUCTION

Onychomycosis is the term used to describe the fungal infection of nail units and onycholysis is described as painless separation of nail plate from its nail bed. It is reported to have an incidence of 0.5 - 5% population worldwide and represents 20-40% of onychopathies , 30% of mycocutaneous infections[1,2] .Several factors have been implicated increase in disease such as reduced peripheral circulation, diabetes, occlusive foot wear, nail trauma, immunosuppressive and antibiotic therapies[3] . Although not life threatening, as it often becomes a chronic source of recurrent infections, besides causing considerable disfigurement.

Diagnosis requires clinical suspicion along with direct (KOH mount) examination with culture. Organisms causing onychomycosis are – Dermatophytes, Nondermatophytes and *Candida albicans* which prevail in fingernail infections. The previous treatment was mechanical or chemical avulsion of nail followed by topical therapy which was unsatisfactory because of remission of disease. Therefore, newer oral antifungal therapy was introduced showed increased recovery rates (Hay 1993)[4]. In view of chronicity, reoccurrence and relapses of this disease, antifungal susceptibility testing is done as a part of diagnosis and treatment. By using these susceptibility testing methods, breakpoints and MIC's can be established which are helpful in treating, treatment failure cases.

MATERIALS AND METHODS

100 patients clinically suspected to have onychomycosis were taken up for this study. Clinical history was taken and a thorough examination of the patients was conducted. Nail scrapings and clippings were obtained from deeper part of discolored or dystrophic parts of nail with the help of scalpel or scissors as the fungus is non-viable in the superficial or distal parts of nails.

Specimen is also collected from nail bed[5]. In females and in others suspected candidal onychomycosis, specimen is collected from proximal and lateral edges with closest proximity[5]. 3 samples were collected from each patient and processed. All samples were subjected to direct microscopy (40% KOH) and for culture, all samples were inoculated onto three sets of Sabouraud's dextrose agar with cycloheximide and Sabouraud's dextrose agar with cycloheximide and chloramphenicol and incubated at 25˚C and 37˚C and observed for growth for six weeks; after which if no growth was observed, sample was reported as culture negative. All samples which were microscopically positive yielded growth on culture. All samples which were KOH negative were also culture negative.

***IN VITRO* SUSCEPTIBILITY TESTING OF ISOLATES**

Antifungal susceptibility testing of yeasts by disc diffusion method[3,6]:-

Medium used is Muller-Hinton agar with 2% glucose and 0.5µg/ml methylene blue, a commercial Hi-media preparation M – 1825(GMB-MHA medium) in accordance with NCCLS (National Committee for Clinical Laboratory Standards M44A guidelines). Antifungal agents tested were Amphotericin B, Ketoconzole, Itraconazole, and Fluconazole. ATCC (American Type Culture Collection) control strain of *C. albicans* – 90028 is used for interpretation of antifungal sensitivity testing.

***In vitro* susceptibility testing of moulds[8]**

NCCLS M38A based broth Macro dilution method. Moulds tested are Aspergillus species, Fusarium species. Medium used is a completely synthetic medium RPMI 1640 with glutamine, without bicarbonate, and with MOPS (3-morpholino propanesulfonic acid) buffer. Final pH of the medium was 7.0 at room temperature. Antifungal agent used was Ketoconazole with diluents as DMSO.(dimethyl sulfoxide)

Procedure:- 0.1ml of 10x drug concentrations are taken in sterile test tubes. Growth control receives 0.1ml of diluted inoculum + 0.1ml of drug diluents without antifungal agent .0.9ml of diluted inoculum suspensions are added and incubated for 46-50 hours without agitation and is observed for growth.

Reading results:- MIC (Minimum Inhibitory Concentration) is the lowest concentration of antifungal agent that inhibits growth as detected visually.

Numerical score is given as

- 4 – No reduction in growth
- 3 – Slight reduction in growth or approximately 75% of the growth control.
- 2 – Prominent reduction in growth or 50% of growth control.
- 1 – Slight growth or 25% of growth control.
- 0 – Optically clear or absence of growth.

RESULTS

Out of 100 patients examined 42 were males and 58 females. Out of these 100 patients 49 cases yielded growth on culture (culture positive) and 46 cases were positive in direct examination (KOH positive).Out of 100 patients suspected of onychomycosis, 27 cases were found to be *Candida albicans* positive by both direct microscopy and culture. 4 cases infected with dermatophytes , 12 cases infected with non-dermtophytic moulds , 3 cases of *Trichosporon* spp and 2 *Geotrichium* cases. A male patient yielded Candida *albicans* on culture (KOH negative) and we also found (1) female case was KOH positive but was culture negative. Rest of the cases (50;n=100) were both negative by direct microscopy and culture .

Table 1: Direct microscopy vs. fungal culture in diagnosis of onychomycosis (n=100)

Diagnostic tests	Culture positive	Culture negative	Total
KOH positive	45	1	46
KOH negative	4	50	54
Total	49	51	100

Table 2: Comparison of Toenail and Fingernail isolates in males and females.

Gender	Toenails isolates	Fingernails isolates
Females	Nil	*Candida albicans* (27) *Geotrichium species* (2)
Males	*Trichophyton rubrum* (2) *Fusarium species* (4) *Aspergillus fumigatus* (4) *Aspergillus niger* (1) *Aspergillus terreus* (1)	*Trichophyton rubrum*(2) *Candida albicans* (1) *Trichosporon species* (3) *Aspergillus niger* (1) *Aspergillus terreus* (1)

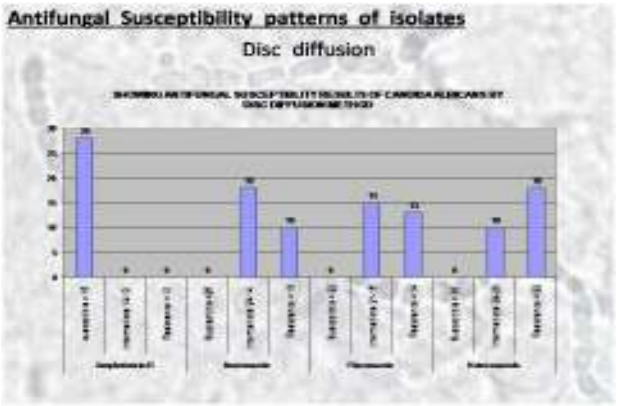

Figure 1: Showing antifungal susceptibility results of Candida albicans by Disc Diffusion method

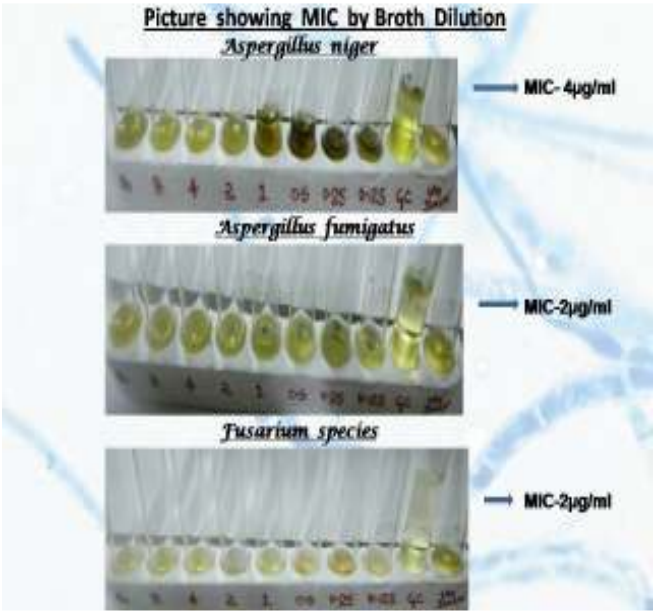

Figure 2: *In vitro* Susceptibility Testing of Culture Isolates by Broth Dilution Method

DISCUSSION

Onychomycosis is an acute or chronic infection of nails and is considered as a serious problem and widely prevalent in all parts of world. Increased prevalence in recent years is attributed to enhanced longevity, comorbid conditions such as diabetes, sports participation, immunocompromised status (HIV), hyperhydrosis, use of commercial swimming pools, wearing of occlusive shoes, nail trauma and old age. This disease is also associated with high rate of recurrences and relapses even after treatment, due to poor penetrability of antifungal agents.

Out of these 100 patients, 46 (46%) cases were positive by both KOH and culture. The KOH positivity rate varied from 35.6-88.6% in various studies like study described by Sanjiv Grover in 2003 and culture positivity rate from 36-54%[9] . Our KOH positivity rate (46%) and culture positivity rate (49%) fell within the reported range.

Common aetiological agents isolated were *Candida albicans*, followed by nondermatophtes and dermatophytes.Dermatophytes are most commonly associated with fingernail and toenail infections and also increasing in HIV infected and other immunocompromised persons, correlating with studies by Kyung Jin Lee et al[10] . Nondermatophytes are most commonly associated with toenails, nail trauma as major predisposing factor followed by diabetes and occlussive footwear which is correlating with the studies made by Sung Min Hwang et al and Amar Surjushe et al[11,12] .

Candidal onychomycosis is most commonly seen in fingernails correlating with the studies made by Ravinder Kaur et al,A.R. Khosravi et al,D.Vijaya et al , SD Rao et al[1,3,13,14] The fingernail infections were commonly reported in women with possible contributing factors like frequent immersion of hands in water and soap.In our present study, we compared the aetiological agents among males and females. Females particularly housewives, launderers of age group 21-40 years were commonly affected with candidal onychomycosis.*Candida albicans* was the predominant isolate, most of these strains were susceptible to Amphotericin B followed by intermediate/dose dependent susceptibility to itraconazole, fluconazole and resistant to ketoconazole.

In case of males, both fingernails and toenails were involved, but non- dermatophytes were isolated more from toenails and showed an MIC of 4µg/ml with ketoconazole. *Trichophyton rubrum* and *Trichosporon species* were isolated from fingernails of immunocompromised individuals.

Antifungal susceptibility results of *Candida albicans* isolates showed 100% sensitivity to Amphotericin B which correlated with the study conducted by Khosravi et al , intermediate/dose-dependent sensitivity to itraconazole, Fluconazole and Ketoconazole. *Aspergillus fumigatus* and *A. niger* showed MIC of 4.0µg/ml and *Fusarium* showed an MIC 2.0µg/ml, when tested with Ketoconazole[3] .

CONCLUSION

In conclusion, our study shows that, fingernails in females were more infected with *Candida species*, microtrauma being most common cause and often associated with paronychia and nondermatophytes were predominant in toenail infections associated with occlusive footware.

Our study shows that infections with dermatophytes and yeasts other than *Candida* were seen in immunocompromised persons, with no particular predilection. Our study also shows that Candidal onychomycosis responds well to topical antifungals in initial stages and Ketoconazole is still effective when given in high doses, can be the drug of choice when Fluconazole is contraindicated and thus avoiding switching onto higher antifungals in this drug resistant era.

REFERENCES

1. Kaur R, Kashyap B, Bhalla P; Onychomycosis – Epidemiology, Diagnosis and Management ; Indian Journal of Medical Microbiology, 2008; 26(2): 108-16

2. Ravinder Kaur, Bineeta Kashyap, Rati Makkar; Evaluation of Clinicomycological Aspects of Onychomycosis, Indian J Dermat, 2008:53(4): 174-8.
3. Khosravi A.R., Shokri H., Mansouri P., Katiraee F., Ziglari T, Candida species isolated from nails and their invitro susceptibility to antifungal drugs in the Department of Dermatology (University of Tehran, Iran), Journal de Mycologie Medicale 2008.
4. Chapter-13, The Dermatophytes, Arvind A., Padhye and Richrd c., Summer bell, Topley & Wilson's Medical Mycology, 10th edition, Edited by William G. Merz and Roderick J. Hay
5. Bertrand Favre, Bettina Hofbauer, Kwang-Soo Hildering and Neil S. Ryder; " Comparision of In Vitro Activities of 17 Antifungal Drugs against a Panel of 20 Dermtophytes by Using a Microdilution Assay ; Journal of Clinical Microbiology, 2003; 4817-4819
6. Chapter-10, Dermatophytoses - Jagdish Chander – Textbook of Medical Mycology; 3rd edition.
7. Method for Antifungal Disc Diffusion Susceptibility Testing of yeasts; Proposed Guideline. CLSI document M44A. CLSI, Pennysylvania, USA 2003
8. Reference method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved standard. CLSI document M38-A. CLSI, Pennysylvania, USA 2002
9. Sanjiv Grover; "Clinico-mycological evalution of onychomycosis at Bangalore and Jorhat" ; Indin J Dermatol Venereol Leprol July-August 2003 ;6(4)
10. Kyung Jin Lee MD, Young Lee MD, Baik KEE, Cho MD, Jong SOO, Choi MD, Hyun Jeong Park MD, Proximal subungual onychomycosis in a patient with Classic Kaposi Sarcoma caused by Trichophyton rubrum; Ann Dermtol, 2011; 23: 1.
11. Sung Min, Hwang MD, Moo Kyu, Suh MD, Gyoung Yim, Ha MD, Onychomycosis Due to Nonder-matophytic Molds; Ann Dermatol, 2012; 24: 2.
12. Amar Surjushe, Ratnakar Kamath, Chetan Oberai, Dattatray Saple, Minal Thakre, Sujatha Dharmshale, Aruna Gohil; A clinical and mycological study of onychomycosis in HIV infection; Indian J Dermatol Venereol Leprol, 2007; 73: 6.
13. Vijaya D, AnandKumar BH., Geetha SH.; Study on onychomycosis; Indian J Dermatol Venereol Leprol, 2004; 70: 3.
14. Rao SD, Wavare S, Patil S, Onycholysis caused by Candida krusei; Indian Journal of Medical Microbiology, 2004: 22(4): 258-259.

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