

## Susceptibility of *Trichophyton mentagrophytes* Against Two Antifungals: Griseofulvin and Terbinafine.

Widad Ismail Thallaj<sup>\*1</sup> & Thekra Ahmed Humada<sup>\*\*</sup>

\*General Health Lab/Kirkuk Health Directorate \*\*Microbiology Branch-Medicine College/Tikrit University

### ABSTRACT

#### Key Words:

*T.mentagrophytes* ,  
Griseofulvin  
,Terbinafine

#### Correspondence:

Widad I. Thallaj  
General Health  
Lab/Kirkuk Health  
Directorate /  
Kirkuk/ IRAQ.

After conventional diagnosis, laboratory experiments were done to assess the effect of griseofulvin and terbinafine on the growth of three isolates of *Trichophyton mentagrophytes* which belong to the genus *Trichophyton* using agar dilution method. Statistically, results of this study revealed significant difference between the effect of terbinafine and that of griseofulvin both against growth of *T.mentagrophytes* isolates where the tested fungi were resistant to griseofulvin in spite of increasing the used concentration and giving means of diameters 21.00 mm and 19.33 mm under effects of 5µg and 60µg concentrations respectively while it revealed more response to terbinafine giving means of diameters 10.6 mm and 0.0 mm under effect of 0.25µg and 2µg respectively. The mean of colony diameter for each concentration of griseofulvin was still same statistically showing no decrease while in case of terbinafine ,the mean of colony diameter differ according to each concentration reaching to complete inhibition.

حساسية الشعرويات الجوزية والترينافين *Trichophyton mentagrophytes* للمضادين الفطريين الجريزوفلفين

وداد اسماعيل ثلاج<sup>\*</sup> و ذكرى احمد حمادة<sup>\*\*</sup>

\*مختبر الصحة العامة / دائرة صحة كركوك \*\* فرع الاحياء المجهرية-كلية الطب/ جامعة تكريت

### الخلاصة

بعد التشخيص الروتيني نفذت سلسلة من التجارب المختبرية لتقييم تأثير عقاري الجريزوفلفين والترينافين في نمو ثلاث عزلات من الشعرويات الجوزية *Trichophyton mentagrophytes* العائدة لجنس *Trichophyton* وذلك باستخدام طريقة تخفيف الأكار . إحصائياً أظهرت نتائج هذه الدراسة وجود فروقات معنوية بين تأثير الترینافین والجريزوفلفين في نمو العزلات الثلاث حيث كانت مقاومة للجريزوفلفين على الرغم من زيادة التركيز معطيةً معدلات أقطار ٢١،٠ ملم و ١٩،٣ ملم تحت تأثير ٥ مايكروغرام و ٦٠ مايكروغرام من العقار على التوالي في حين أظهرت إستجابة أكثر تجاه الترینافین معطيةً معدلات أقطار ١٠،٦ ملم و ٠،٠ ملم تحت تأثير ٠،٢٥ مايكروغرام و ٢ مايكروغرام من العقار .

الكلمات المفتاحية:  
الشعرويات الجوزية ،  
الجريزوفلفين ، الترینافین .  
للمراسلة :  
وداد اسماعيل ثلاج  
مختبر الصحة العامة /  
دائرة صحة كركوك /  
كركوك / العراق .

### Introduction:

Dermatophytes are groups of fungi having ability to invade the superficial layer of the epidermis and digest the keratinized tissues of skin, hair and nails in living animals including man(Yadav *et al*,2013).The dermatophytes have been classified into 3 genera: *Trichophyton* (infects hair, skin and nails),*Microsporum* (infects hair and skin), and *Epiderophyton*(infects skin and nails)(Kokare,2008).The genus *Trichophyton* is particularly important and represents the most frequently isolated from human patients.Apart from the species present in this genus,*T.mentagrophytes* contain several different variants which occur in both animals and man(Liu

<sup>1</sup> This paper is a part of M.Sc. thesis for the first author

*et al*,1997).*T.mentagrophytes* is an important dermatophytic pathogen that can cause onychomycosis, tinea corporis and tinea pedis. It has been reported that 10% of the total number of cases of dermatophytosis are caused by *T.mentagrophytes*(Zhang *et al*,2014).In many area of the world *Trichophyton* is a major concern as it is responsible for feet and nails infections that are extremely difficult to cure (Blumer ,1995).It is important to perform antifungal susceptibility testing to provide information that allow clinicians to select appropriate antifungal agents useful for treating a particular fungal infection and to evaluate the resistant dermatophytes and to determine the antifungal activity(Yadav *et al*,2013).Griseofulvin is preferentially used for the treatment of infections caused by dermatophytic fungi like *Epidermophyton*, *Trichophyton*, and *Microsporum*.Because of its capacity to concentrate in the keratinous layer of the epidermis and its relatively low toxicity in man, griseofulvin is used in the therapy of cutaneous mycoses like tinea capitis and tinea corpori in the children by oral administration.Several dermatophytic fungi like *T.mentagrophytes*, *M.gypseum* have been reported to develop resistance to griseofulvin *in vivo* and *in vitro* after treatment with mutagens(Esser and Bunnett,2002).Terbinafine has a rather broad spectrum of activity that includes dermatophytes,*Candida spp*,*Malassezia furfur*.It exhibits low toxicity and good efficacy in the treatment of virtually all dermatomycoses(Victor,2005).This study aims at determining susceptibility of *T.mentagrophytes* to griseofulvin versus terbinafine at different concentrations.

## **Materials and Methods:**

### **Isolation and Identification of The isolates:**

Three isolates of *T.mentagrophytes* were used in this study. These were isolated from patients attending Dermatology Consultation on Kirkuk General Hospital and suffering dermatological infections. Skin scraps obtained from the patients were cultured on Sabouraud Dextrose Agar after they had been identified as fungal infections using 10%KOH.Growth were identified by conventional method used by(Nakamura *et al*,2002).

### **Antifungal Susceptibility Testing:**

Griseofulvin and Terbinafine antifungal drugs were selected and tested for thier activities. Stock solutions were prepared by dissolving 500 mg tablet of griseofulvin(Laboratories pharma-Morocco) in 5 ml 100% Dimethyl Sulfoxide and dissolving 250 mg tablet of terbinafine (Laboratories pharma-Morocco) in 5 ml of 95% ethanol as stock solutions from them required concentrations were prepared and the rest kept at 4°C for future use .

### **Determination of Fungal Growth:**

For obtaining various concentrations, antifungal was diluted with melted prepared Potato Dextrose Agar media (5,10,20,30,40,50,60 µg/ml for griseofulvin and 0.25, 0.5, 1,2 µg/ml), then poured in sterilized petri dishes. A disk (7mm) of old grown fungi(at 28°C for 2 weeks)was inoculated on the center of culture media. Plates were incubated at 28°C for 2 weeks. Perpendicular colony diameters(mm) of grown isolates were measured. Triplicates were done for each isolate in every concentration of the antifungal(Muharrem ,2013).

## **Results and Discussion:**

Growth appeared on cultured media after 18 days at 28°C .Colonies of *T.mentagrophytes* were flat white to cream in color, with powdery to granular surface(figure 1).Reverse pigment was reddish-brown color. Microscopically, Lactophenol cotton blue stain slides of the growth revealed numerous microconidia arranged in clusters with smooth thin walled clavate multicelled macroconidia(figure 2).Spiral hyphae were also present(figure 3). As well as the present study revealed that *T.mentagrophytes* was positive for hydrolysis of urea(Issa and

Zanganz,2009).Development of specialized perforating organs invading hairs and engendering conspicuous conical pits at right angles to the long axis of the hair were seen in hair perforation test (figure 4) which was confirmatory for *T.mentagrophytes* as indicated by(Weitzman and Summerbell,1995).



Figure 1: Colony of *T.mentagrophytes*

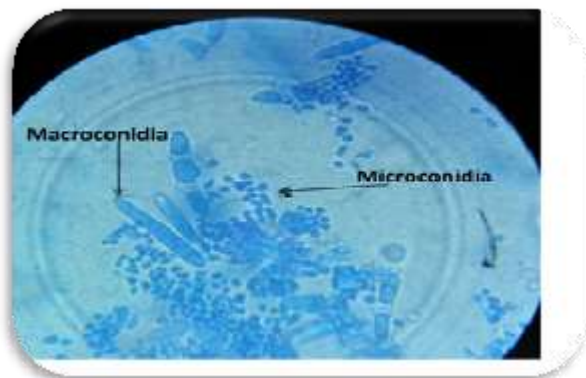


Figure2:Macro- and Microconidia of *T.mentagrophytes*

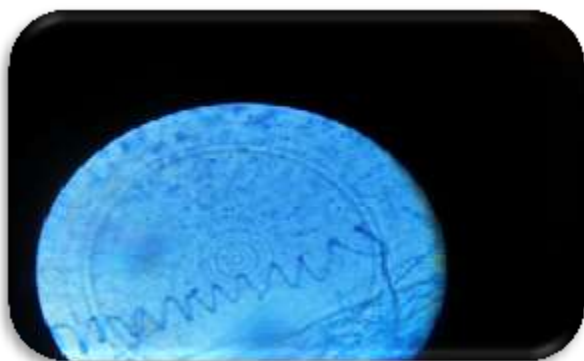


Figure 3: Spiral hyphae of *T.mentagrophytes*

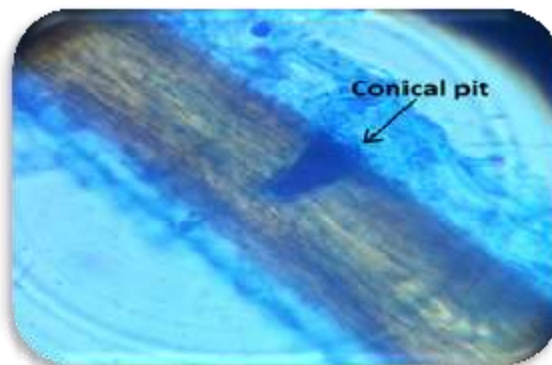


Figure 4:Positive hair perforation test in *T.mentagrophytes*

Results of susceptibility test as showed in table 1 revealed that there was no significant difference in the means of diameters for the concentrations of griseofulvin and this was statistically approved at  $p < 0.005$ . From the table it can be seen that at  $5\mu\text{g/ml}$ (figure 5 ), the mean of the diameter for the three isolates was 21.00 mm. With increasing griseofulvin concentration reaching the final tested concentration  $60\mu\text{g/ml}$ (figure 6 ), the mean of diameter was 19.33 mm which was not differ from that of  $5\mu\text{g/ml}$  meaning that the three isolates resisted griseofulvin even in case of increasing the concentration. Regarding terbinafine effect on the growth of three isolates of *T.mentagrophytes* , it was differ completely from that of griseofulvin where the mean of diameter at

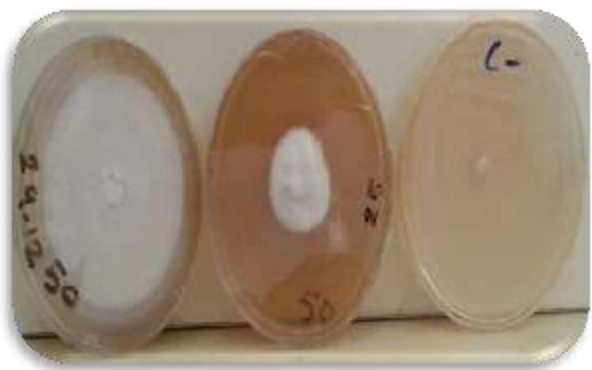


Figure5: Effect of 5µg Griseofulvin  
(from left: ctrl +ve,test,ctrl-ve)

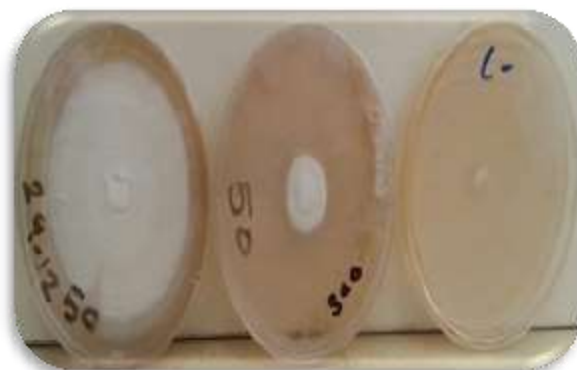


Figure 6 :Effect of 60µg Griseofulvin  
(from left:ctrl+ve.test,ctrl-ve)



Figure 7: Effect of 0.25µg Terbinafine  
(from left:ctrl+ve,test,ctrl-ve)

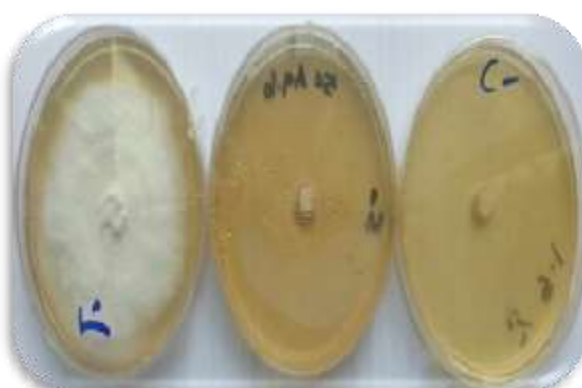


Figure 8: Effect of 2 µg Terbinafine (from  
left:ctrl+ve,test ,ctrl-ve)

the first used concentration(0.25µg/ml) of terbinafine was 10.6 mm (figure 7) and this was significantly different from the mean of diameter at the final tested concentration(2µg/ml) that was 0.0 mm (figure 8) and produced complete inhibition of the growth . Means of diameters of the three isolates were compared to the mean of diameters of three control positive used which was 61.6 mm and that of three control negative which was 0.0 mm.

These results were in accordance with that of (Fernandez-Torres,2001;Santos *et al*, 2006).*In vivo*, resistance is also correlated with antifungal misuse because patients often fail to finish the full course of treatment(Martinez-Rossi,2008). From the present study, it can be concluded that Terbinafine is more potent antifungal agent (agreeing with results of Girgis *et al*,2006) than griseofulvin,so it is important to prescribe griseofulvin more carefully in treating dermatophytosis.

Antifungal susceptibility testing is a dynamic field of medical mycology. Agar based method enables the determination of the activity of various antifungal drugs against various fungal genera and species(Pakshir *et al*,2009).The determination of fungus *in vitro* antifungal susceptibility has been reported to be important for the ability to eradicate pathogenic dermatophytes(Ebrahim *et al*,2010 ).

**Table 1: Effect of two antifungals(Griseofulvin and Terbinafine) on growth of the three isolates of *T.mentagrophytes***

Antifungal agent	Conc. µg/ml	Colony diameter(mm)			Mean Diameter for the Conc.(mm)
		Isolate 1	Isolate 2	Isolate 3	
Griseofulvin	5	32 d	16 hi	15 hi	21.00 ±9.54 B
	10	30 d	19 gh	15 hi	21.33 ±7.77 B
	20	26 e	23 ef	18 gh	22.33 ±4.04 B
	30	23 ef	25 e	16 hi	21.33 ±4.73 B
	40	23 ef	20 fg	20 fg	21.00 ±1.73 B
	50	24 ef	18 gh	13 ij	18.33 ±5.51 B
	60	21 fg	21 fg	16 hi	19.33 ±2.89 B
<b>Mean of Colony Diameter</b>		25.57 ±4.04 aB	20.29 ±3.04 bB	16.14 ±2.268 cB	
Terbinafine	0.25	12 ijk	11 jk	9 k	10.667 ±1.528 C
	0.5	0	0	0	0.000 ±0.000 D
	1	0	0	0	0.000 ±0.000 D
	2	0	0	0	0.000 ±0.000 D
<b>Mean of Colony Diameter</b>		3.00 ±0.71 aC	2.75 ±0.33 aC	2.25 ±0.31 aC	
<b>Control positive</b>		75 aA	49 cA	61 bA	61.67 ±13.01 A
<b>Control negative</b>		0 D	0 D	0 D	0.00 ±0.00 D

\*Same capital letters in the same column means no significance differences( at p≤0.05).

\*Same small letters means no significance differences (at p≤0.05).

### References:

- Bulmer G.S.(2005).Fungus disease in the orient.3<sup>rd</sup> ed.Philipne:Rexbook store ;89-96.  
 Ebrahim H.M ,Asaad A.M ,Amer A.Antifungal susceptibility patterns of dermatophytes clinical isolates from dermatophytosis patients before and after therapy.*Egyp.J. of Med.Microbiol* 2010 ;19(1):41-46.  
 Esser K.&Bennett J.(2002).The mycota:Industrial applications.Printed in Germany: Springer;94.  
 Fernandez-Torres B,Carrillo A.J,Martin E.,Del Palacio A.,Moore M.K.,Valverde A.,Serrano M. and Guarro J.(2001).*In vitro* activities of 10 antifungal drugs against 508 dermatophyte strain.*Antimicrobial agents and chemotherapy*;45(9):2524-2528.

- Girgis S.A, Zu El-Fakkar N.M, Badr H, Shaker O.A, Metwally F.E, Bassim H.H .(2006). Genotypic identification and antifungal susceptibility pattern of dermatophytes isolated from clinical specimens of dermatophytosis in Egyptian patients .*Egypt. Dermatology J* ;2(2):1-23.
- Issa N.A and Zangana I.K.(2009). Isolation of *T. mentagrophytes* var *mentagrophytes* from naturally infected laboratory albino rats: experimental infection and treatment in rabbits. *Iraqi J. of vet sci*;23(2):29-34
- Kokare Ch.R.(2008). Microbiology for nursing and health science. 1<sup>st</sup>.ed. Nirali-Parkashan ;7.41-7.43.
- Liu D, Coloe S, Baird R, and Pedersen J.(1997). PCR identification of *T. mentagrophytes* var *interdigitale* and *T. mentagrophytes* var *mentagrophytes* dermatophytes with a random primer. *J. Med. Microbiol*;46:1043-1046.
- Martinez-Rossi N.M, Peres N.T, and Rossi A.(2008). Antifungal resistance mechanisms in dermatophytes. *Mycopathologia*;166:369-383.
- Muharrem T.(2013). Antifungal effect of Various Salts Against *Fusarium oxysporum* f.sp. *cepae* ,the causal agent of *Fusarium* Basal Rot of Onion. *Journal of Agricultural Science*;19:178-187.
- Nakamura Y, Kano R , Hasegawa A , Watanabe Sh.(2002) .IL-8 and TNF- $\alpha$  production in human epidermal keratinocytes induced by *T. mentagrophytes*. *American society for Microbiology*;9(4):935-937.
- Pakshir K, Bahaedinie L, Rezaei Z, Sodaifi M, Zomorodian K.(2009). *In vitro* activity of six antifungal drugs against clinically important dermatophytes. *Jundishapur journal of Microbiol*;2(4): 158-163.
- Santos D.A, Barros E.S, Hamdan J.S.(2006). Establishing a method of inoculum preparation for susceptibility testing of *T. rubrum* and *T. mentagrophytes*. *J. of Clin. Microbiol*;44(1):98-101.
- Victor L.(2005). Antibiotics in laboratory medicine 5<sup>th</sup> ed. USA: Lippincott William & Wilkins;239.
- Weitzman I. and Summerbell R.C.(1995). The dermatophytes. *Cli. Micobiol. Rev*;8(2):240
- Yadav A, Urhekar AD, Mane V, Danu M.S, Goel N, KG A.(2013). Optimization and isolation of dermatophytes from clinical samples and *in vitro* antifungal susceptibility testing by disc diffusion method. *J. of Microbiol. and Biotech.*;2(3)19-34.
- Zhang X, Wang Y, Chi W, Shi Y, Chen S, Lin D, Jin Y.(2014). Metalloprotease genes of *T. mentagrophytes* are important for pathogenicity. *Medical Mycology*;52:36-45.