ORIGINAL ARTICLE

KOH mount versus culture in the diagnosis of tinea capitis

Hamed Mohammed Abdo[1] MD , Mohammed Rashed Abdel-Hamed[2] MD ,
Ibrahim M Al-Hosiny[3] MD

[1]*Department of Dermatology, Venereology & Andrology, Faculty of Medicine - Al-Azhar University, Cairo, Egypt*
[2]*Department of Clinical Pathology, Faculty of Medicine - Al-Azhar University, Cairo, Egypt*
[3]*Department of Microbiology & Immunology, Faculty of Medicine - Al-Azhar University, Cairo, Egypt*

ABSTRACT

Background: Tinea capitis is a fungal infection caused by dermatophytes which invade the hair shaft, follicle and surrounding scalp skin. The major etiologic agents of tinea capitis vary in different areas. Clinical presentations varied from scaly patches of hair loss, black dot, seborrhoeic dermatitis like, pustules, kerion with associated lymphadenopathy and favus. It is unreliable to depend on clinical diagnosis alone and laboratory methods should be used wherever possible to confirm the diagnosis.

Aim: The present work involved comparison of standard potassium hydroxide (KOH) mount versus mycological culture in the diagnosis of tinea capitis with the aim of identification and isolation of the causal agent.

Patients and methods: A total of 35patients with clinically suspected tinea capitis were selected from attendants to Al-Hussein University Hospital, Cairo, Egypt. Hair and or scale specimens were mounted in 20% KOH and subjected to direct microscopic examination for the presence of fungal elements. Other specimens were inoculated onto Sabouraud's dextrose agar (with and without cyclohexamide).

Results: Direct microscopy with KOH mount was positive in 30/35 (85.7%) while mycological culture showed positive results in 21/35 (60%) of patients. KOH mount has higher sensitivity compared to that of mycological culture (85.7% versus 60%). Causal agents were isolated as follows: *Trichophyton (T) violaceum* in 8 cases, followed by *T. tonsurans* in 4 cases, *T. rubrum* in 4 cases, *T. schoenleinii* in 3 cases and *T. verrucosum* in 2 cases.

Conclusion: KOH mount was more sensitive than mycological culture. It was easy to perform, rapid, and gave significantly higher rates of positivity compared to the mycological culture.

KEYWORDS: Potassium hydroxide mount, mycological culture, tinea capitis

INTRODUCTION

Tinea capitis is a worldwide public health problem that affects children bellow 15 years of age and requires identification of the specific causative fungal agent. It is characterized by infection of the hair and skin of the scalp associated with symptoms and signs of inflammation and hair loss. The predominance of specific pathogens causing tinea capitis varies with geography, environments, climates, occupations, ethnic groups and life styles.[1, 2, 3]

The etiologic agents originate from different sources. Based on host preference and natural habitat, these agents are classified into three categories: anthropophilic, zoophilic, and geophilic species. Anthropophilic species usually infect humans. Zoophilic species are pathogens of animals rather than human and transmission from animals to humans can occur. Geophilic species inhabit soil and infect both humans and animals. Some species are harbored and transmitted by fomites.[3] Dermatophyte infection of the scalp can usually be diagnosed clinically. Additional diagnostic methods include a potassium hydroxide (KOH) preparation and microscopy, culture on Sabouraud's dextrose agar (SDA), Wood's lamp examination and skin biopsy.[4]

Correspondence: *Dr. Hamed Mohammed Abdo , Department of Dermatology, Venereology & Andrology, Faculty of Medicine - Al-Azhar University, Cairo, Egypt , E-mail: hamed392@yahoo.com*

AIM OF THE STUDY:

The present study involved comparison of standard KOH mount versus mycological culture in the diagnosis of tinea capitis with the aim of identification and isolation of the causal agent.

PATIENTS AND METHODS

Patients: A total of 35 patients (19 males and 16 females) with clinically suspected tinea capitis were selected from attendants to Al-Hussein University Hospital at Cairo, Egypt. Their ages ranged from 2 to 21 years (mean 5.2 years). Patients that have received systemic antifungal treatment for the last four weeks and/or topical antifungal medications for the last two weeks were excluded.

Methods: In suspected cases, after cleaning the selected area with alcohol, dull lusterless hairs and hair stubs were plucked by sterile forceps to include the hair roots. Scaly patches on the scalpwere also scrapped with the blunt edge of the scalpel.

Potassium hydroxide mount

Scales and plucked hair stubs were used for examination. Scales were gently scraped off by the blunt edge of a number 15 blade onto a clean glass slide. Hairs were plucked by sterile forceps to include the hair roots. A drop of 20% KOH was placed next to the material and then thoroughly mixed and a cover slip was applied. The preparation was left for 20-60 minutes until softening and digestion of the specimen occurred. Slides were evaluated for the presence of fungal elements under a microscope (x100, x200 and x400) magnification. The presence of fungal hyphae and/or spores within (endothrix) and/or around (ectothrix)
hair shafts was considered to be a positive test).

Culture on Sabouraud's dextrose agar (SDA)

After cleaning the selected area with alcohol, materials were collected as for KOH mount using sterilized instruments. Scales, crusts and/or hair stubs were inoculated onto two types of culture media: SDA with cycloheximide (to suppress the growth of contaminant fungi) and SDA without cycloheximide. Chloramphenicol was added to both culture media (to prevent bacterial overgrowth). The media were then incubated in a warm, moist environment at 28 °C and examined regularly to detect growth of any fungus. Observation for growth was done periodically for at least 4 weeks after which the media were reported as positive or negative. The fungi were identified by noting their growth rate, colonial morphology, and microscopic structures. Colonial morphology includes color, size, texture, and topography of the colony. The microscopic structures of fungi usually provide definitive identification. Microscopic features that were looked for are the type, size, shape and arrangement of spores and the size and color of hyphae.

RESULTS

Direct microscopy with KOH mount was positive in 30 cases out of 35 cases (85.7%) while mycological culture showed positive results in 21cases out of 35 (60%) patients. KOH-positive cases showed Endothrix in 15 cases (Fig. 1), ectothrix in 7 cases (Fig. 2) and endo/ectothrix type in 5 cases (Fig. 3). Favic type of hair invasion has been observed in 3 cases (Fig. 4) . The results of KOH mount were summarized in Table-1. All KOH-positive cases were also culture-positive except in one case. KOH mount has higher sensitivity compared to that of mycological culture (85.7% versus 60%). Causal agents were isolated as follows: *Trichophyton (T) violaceum* in 8 cases (Fig. 5 a and b), followed by *T. tonsurans* in 4 cases, *T. rubrum* in 4 cases, *T. schoenleinii* in 3 cases and *T. verrucosum* in 2 cases (Table 2). Out of 35 cases of tinea capitis, 19 were males and 16 were females. Age ranged from 3 -27 yrs (mean-7.6 yrs) and 32 were children while 3 were adults. Scaly type lesions were observed in 12 cases while black dot lesions were present in 9 cases and in 1 case, kerion type lesion was observed. In 3 adult cases, favus type lesion was observed while the remaining patients showed variety of clinical picture. Duration of disease ranged from 1 month to 3 years (mean 2.9 months).
Clinical data are summarized in Table 3.

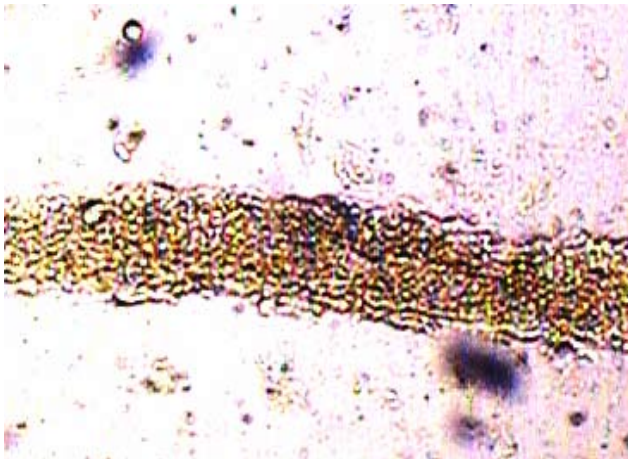

Fig. 1 Endothrix type of hair invasion by *T. violaceum* (KOH mount x 200).

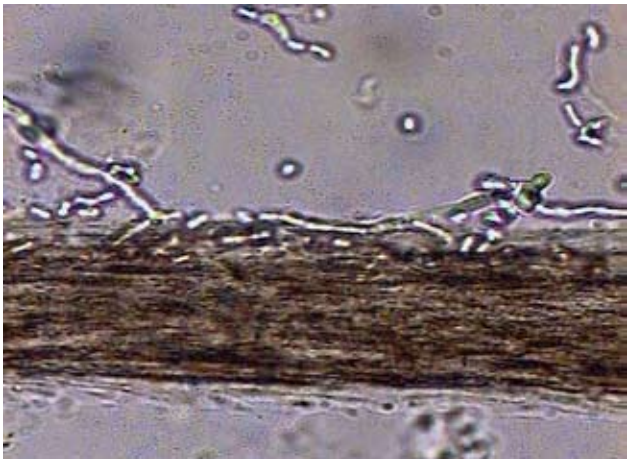

Fig. 2 Ectothrix type of hair invasion by *T. verrucosum* (KOH mount x 400).

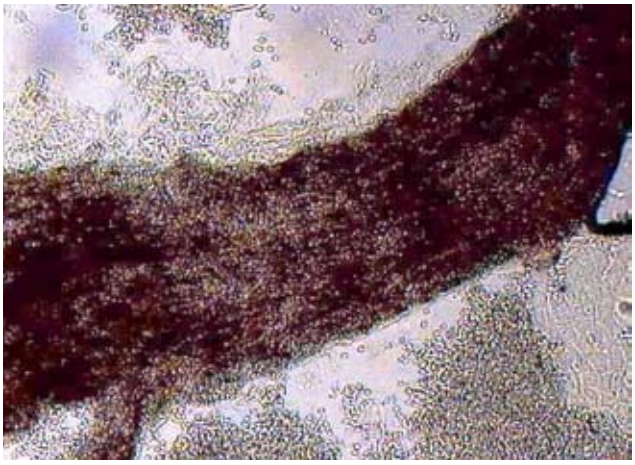

Fig. 3 Both endothrix/ectothrix type of hair invasion caused by *T. rubrum* (KOH mount x 200).

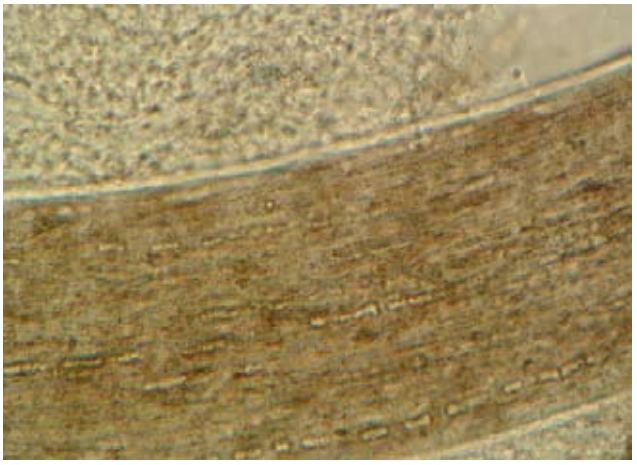

Fig. 4 Favic type of hair invasion characteristic to *T. schoenleinii* (KOH mount x 400).

Fig. 5 a. Macroscopic morphology of T. violaceum showing folded deep-violet colonies.

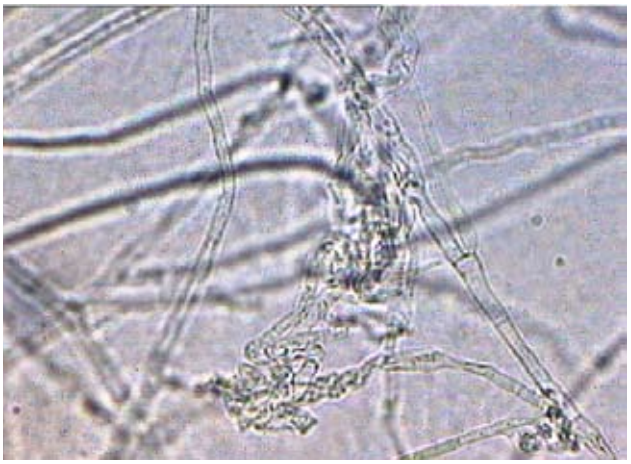

Fig. 5 b. Microscopic morphology of *T. violaceum* showing characteristic tortuous, broad, distorted and much-branched hyphae with lack of conidia.

DISCUSSION

Reported sensitivity of fungal culture for identifying dermatophytes varies from 25% to 80% with approximately 30% false-negative results with culture and KOH studies[5]. In this study, the sensitivity of KOH was 85.7% while that of fungal culture was 60%. In agreement with this result, Tandon et, al, (1996)[6] reported that from 298

Table 1 Results of KOH mount

KOH mount *(no. 35)*					
Positive cases			**Negative cases**		**KOH sensitivity**
No.	*%*		*No.*	*%*	
30/35	85.71%				
Pattern of hair invasion					
Pattern	*No.*	*%*			
Endothrix type	15	50.00%	5/35	14. 28%	85.71%
Ectothrix type	7	23.33%			
Endo/ectothrix type	5	16.66%			
Favic type	3	10.00%			

Table 2 Results of fungal culture

Fungal culture *(no. 35)*					
Positive cases			**Negative cases**		**Culture sensitivity**
No.	*%*		*No.*	*%*	
21/35	60%				
Isolated agents					
Name	*No.*	*%*			
T. violaceum	8	38.09%	14/35	40%	60%
T. tonsurans	4	19.04%			
T. rubrum	4	19.04%			
T. schoenleinii	3	14.28%			
T. verrucosum	2	9.52%			

Table 3 Clinical data

Sex distribution		**Age distribution**	**Duration of disease**	**Clinical type *(no 35)***	
Males	*Females*	Range 3 -27 yrs		*lesion*	*No.*
19	16	Mean = 7.6 yrs	Range 1 month - 3 yrs	Scaly type	12
		32 cases < 14 yrs	Mean = 2.9 months	black dot	9
		3 cases > 14 (17, 21, 27) yrs		favus type	3
				kerion	1
				others	10

clinically diagnosed cases, 65.43% were KOH positive and 46.97% showed culture growths of pathogenic dermatophytes.

Shenoy et, al, (2008)[7] also proved that KOH mount and mycological culture showed positive results in 53% and 35% of patients respectively. In addition, out of 174 specimens, Ali et, al, (2009)[1] revealed that 65.5% had fungal elements on KOH mount while 50% were culture positive. These results, hence, document that mycological culture was less sensitive than KOH mount. However, KOH mount is unable to identify and isolate the causative dermatophytes as does fungal culture.

Dermatophytes are known to grow best in warm and humid environments and are, therefore, more common in tropical and subtropical regions and this probably explains why they are very common in Africa. Some species of dermatophytes such as T. mentagrophytes, Microsporum canis and T. rubrum are distributed all over the world. However, other species probably have partial geographic restriction.[8] T. schoenleinii is found in Africa and Eurasia while T. soudanense is restricted within Africa.[9] Others as T. violaceum is associated to Asia, Africa and Europe and T. concentricum is known to be common in the Far East, India and the Pacifics.[10]

In this study, causal agents were isolated as follows: T. violaceum in 8 cases, followed by T. tonsurans and T. rubrum (4 cases each), then T. schoenleinii (3 cases) and T. verrucosum (2 cases). Jin et, al, (2005)[3] reported that T. violaceum was the major etiological agent, followed by T. verrucosum and T. tonsurans. Infections due to T. schoenleinii and T. rubrum (which isolated in this work) were not detected in their study. Also from 174 specimens, Ali et, al, (2009)[1] identified 86.2% as T. violaceum and 13.8% as T. verrucosum.

On the other hand, Marques et, al, (2005)[11] isolated M. canis in 88.2% of their cases, followed by T. tonsurans (4.7%), T. rubrum (3.3%), M. gypseum (1.9%) and T. mentagrophytes (1.6%). In disagreement with this work, T. violaceum and T. schoenleinii were not recovered in Marques et, al study although it was carried out on large number (1055) of suspected cases, while M. canis, M. gypseum and T. mentagrophytes were not recovered in this study. T. schoenleinii, the causative agent of favus, is endemic in certain areas in Egypt so we were able to isolate it while in Marques et, al region, favus may not be an endemic infection.

This difference in dermatophyte isolation could also be explained by different samples of study population and again emphasize the role of different environmental conditions and degree of exposure to the pathogens. The predominance of certain dermatophytes causing tinea capitis varies geographically depending on different environmental factors such as climates and humidity, occupations, ethnic groups and different life styles.

In this work, we were able to isolate both zoophilic (T. verrucosum) and anthropophilic (T. violaceum, T. tonsurans, T. rubrum and T. schoenleinii) fungi from patients living in urban area which could be explained by the process of immigration and traveling from rural to urban regions and vice versa. Zoophilic species are pathogens of animals which known to produce inflammatory tinea capitis, however; the zoophilic T. verrucosum was isolated in this study, unexpectedly, from scaly non inflammatory lesion. So, one must keep in mind that zoophilic fungi can produce both types of lesions.

In this study, of the 35 cases of tinea capitis, 19 were males and 16 were females with 32 were children below the age of 14 years and 3 were adults. Scaly type lesions were observed in 12 cas-

es while black dot lesions were present in 9 cases and in 1 case, kerion type lesion was observed. In 3 cases aged over 17 years, favus type lesion was observed while the remaining patients showed variety of clinical picture. Comparable with these findings, Mankodi and Kanvinde, 1968 [12] reported on 30 cases of tinea capitis that 28 were children below the age of 13 years and two were adults. Sex incidence showed that 21 were males and 9 were females. Typical "Blackdot" lesions were present in 13 cases like others showed variety of clinical picture. In one case kerion type lesion was observed.

In this work, all KOH-positive cases were also culture-positive except in one case. This KOH-positive culture-negative case could be explained, after excluding the possibility of KOH-interpretation error, by 1) the inoculated fungi were non viable, 2) fungi may have died during sample processing or culture, 3) the inoculated fungi were suppressed by cyclohexamide but the inoculation was done on both types of media (with and without cyclohexamide), and 4) the inoculated sample was not enough to include the causative fungi or was chosen from non optimal site. For all these reasons, a fungal culture should be repeated in suspected cases when a negative culture is observed and a KOH preparation is positive.

CONCLUSION

KOH mount was more sensitive than mycological culture. It was easy to perform, rapid, and gave significantly higher rates of positivity compared to the mycological culture. However, owing to the possibility of false negative (missed) cases of both procedures, both should be a complementary for each other in the diagnosis of suspected cases. Whenever possible, fungal culture should be repeated in suspected cases when a negative culture is observed and a KOH preparation is positive.

REFERENCES

1. Ali J, Yifru S,Woldeamanuel Y. Prevlence of tinea capitis and the causative agent among school children in Gondar, North West Ethiopia. Ethiop Med J 2009; 47: 261-9.
2. Hay RJ, Moore M. Mycology. In: Champion RH, Burton JL, Burns DA and Breathnach SM. Rook's Textbook of Dermatology 6th Edition. Blackwell Science, Oxford 1988; pp 1277-1376.
3. Jin Y, Rouyu L and Glenn B. Current topics of tinea capitis in China. Jpn J Med Mycol 2005; 46: 61-6.
4. Jordaan HF. The diagnosis and management of tinea capitis. SA Pharmaceutical J 2006; 8-11.
5. Suarez SM, Silvers DM, Scher RK, Pearlstein HH, Auerbach R. Histopathologic evaluation of nail clippings for diagnosing onychomycosis. Arch Dermatol 1991; 127: 1517-19.
6. Tandon S, Dewan SP, Mohan U, Kaur A, Malhotra SK. Mycological aspects of dermatomycosis. Indian J Dermatol Venereol Leprol 1996; 62(5): 336-37.
7. Shenoy MM, Teerthanath S, Karnaker VK, Girisha BS, Krishna Prasad MS, Pinto J. Comparison of potassium hydroxide mount and mycological culture with histopathological examination using periodic acid-Schiff staining of nail clippings. Indian J Dermatol Venereol Leprol 2008; 74: 226-30.
8. Nweze EI. Dermatophytosis in Western Africa: A review. Pak J Biol Sci 2010; 13: 649-56.
9. Weitzmann I , Summerbell RC. The dermatophytes. Clin Microbiol Rev 1995; 8: 240-59.
10. Ameen, M. Epidemiology of superficial fungal infections. Clin Dermatol 2010; 28: 197-201.
11. Marques SA, de Camargo RMP, Fares AHG, Takashi RM, Stolf HO. Tinea capitis: epidemiological and ecological aspects of cases observed from 1983 to 2003 in the Botucatu Medical School, state of São Paulo-Brazil. An Bras Dermatol 2005; 80(6):597-602.
12. Mankodi RC, Kanvinde MS. Fungus Infection of Scalp and Hair - a Study of Thirty Cases. Indian J Dermatol Venereol Leprol 1968; 34 : 186-88.