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ORIGINAL ARTICLE

Rapid Detection of Dermatophytes in Primary School Children with Tinea Capitis

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ABSTRACT

Background: Tinea capitis is a dermatophyte infection of the scalp, eyebrows and eyelashes. The disease is common among preadolescent children and mainly caused by Trichophyton and Microsporum species. Accurate and rapid identification is challenging as conventional methods are slow and mostly based on morphological characteristics. ***Objective*** was to investigate the usefulness of nested polymerase chain reaction (PCR) and Dermatophyte Test Medium (DTM) for rapid, accurate & reliable diagnosis of tinea capitis and to identify the most common causative dermatophytes among primary school children. ***Methodology*** One hundred clinical specimens from children pre-diagnosed clinically as tinea capitis were collected and analyzed using conventional mycological methods (culture on SDA and KOH microscopy), culture on DTM and pan-dermatophytes nested PCR**.** ***Results*** 82/100 clinical sample were found positive with pan - dermatophyte nested PCR, followed by culture on DTM which could detect dermatophytes in 78 % of suspected cases. Hyphae and/ or spore structures were observed in 68% of samples with direct KOH microscopy and lastly, dermatophytes were isolated by conventional culture in (56%) of samples. *Microsporum canis* (42.9%) was the most prevalent strain, followed by *T. mentagrophytes* (23.2%). There was good agreement between both nested PCR and DTM with direct KOH microscopy and there was moderate agreement between both methods and conventional culture. However, there was very good agreement between the two investigated methods and our data indicated that the two methods yielded higher positivity in less time than conventional methods. ***Conclusion:*** By the use of nested PCR, rapid reliable results were achieved within 24 hour in contrast to the 4 weeks incubation required by conventional culture. This technique is not only rapid but also simple and inexpensive in comparison to other molecular methods. DTM is ideal for hospital practice as it can be used as a screening test for detection of dermatophytes but additional testing may be needed for species identification.

INTRODUCTION

Tinea capitis is a superficial fungal infection of the scalp skin, eyebrows and eyelashes, with a tendency for invading hair shaft and follicle**.** Dermatophytes are responsible for the majority of superficial fungal infections; they are a group of correlated keratinophilic fungi that can attack keratinized tissues such as skin, hair and nails causing dermatophytosis **[1].**

The clinical presentation of tinea capitis is variable, ranged from a scaly non inflamed dermatosis similar to seborrhoeic dermatitis up to inflammatory disease with hair loss that can progress to deep abscesses named kerion with the opportunity for scarring and permanent alopecia. The type of the disease depends on interaction between the etiologic fungus and the host **[2].**

In Egypt information about the prevalence of tinea capitis is limited; it is more common among African Americans**.** It is responsible for up to 92.5% of dermatophytosis in children younger than 10 years. The disease is rare in adults, although occasionally, it may be found in elderly patients **[3].**

Wood's lamp examination may be benficial for ectothrix infections however; in case with endothrix type, negative Wood's lamp results are of no value for screening or monitoring infections**.** Microscopic revealing of fungal elements from clinical samples is a rapid diagnostic tool nevertheless; it lacks specificity and sensitivity with false negative results in up to 15% of cases adding to; failure in species differentiation. In vitro culture is a specific diagnostic technique however; it is slow, may take up to 4 weeks to give results. In addition, isolates from patients on antifungal therapy do

not yield typical morphology on culture, thus further compromising the results of culture [4].

Nested - PCR is the second round of amplification on a DNA fragment which is already the result of PCR reaction, so decreasing the likelihood of contamination and increasing the sensitivity and specificity of PCR [5].

The aim of the study was to investigate the usefulness of nested - PCR and Dermatophyte Test Medium (DTM) for rapid, accurate & reliable diagnosis of tinea capitis and to identify the most common causative dermatophytes among primary school children.

METHODOLOGY

Study design:

This cross sectional study was carried out at Dermatology & Clinical Pathology Departments of Banha University Hospital during the period from December 2014 to December 2015. A total of one hundred children displaying clinical signs of tinea capitis such as alopecia, patchy hair loss, scaling, or scalp inflammation and their ages ranged from 4 to 11 years were enrolled in the study. They were classified into 3 groups; Group (a): children aged 4 to less than 6 years, Group (b): from 6 to less than 9 years and Group (c): from 9 to 11 years. Children were excluded if they had known dermatological condition such as psoriasis or eczema or had been treated for tinea capitis within the previous period or using any topical medication or suffered from generalized lymphadenopathy. Enrolling procedures included, history taking, general examination and complete dermatological examination. The study design was approved by the local Ethics Committee of Banha faculty of medicine.

Samples collection:

Specimens were collected after cleaning the scalp with 70% ethyl alcohol. Hair was then epilated by using forceps and was collected from the active edge of the lesion and from the center as well together with scales and crusts. Dull, grey or broken hairs were selected and hair stumps were pulled [4].

Laboratory methods

Potassium Hydroxide examination: (20% KOH Solution): Mosaic arrangement of spores was seen on the surface of the shaft (ectothrix infection) or hyphal fragments and arthroconidia was seen internally (endothrix infection) using low and high power of light microscope [6].

Culture on Sabouraud's dextrose agar (SDA- Oxoid): A primary culture was prepared onto SDA and Dermasel agar with chloramphenicol and cyclohexamide. The plates were incubated for at least 4 weeks at room temperature and assessment was performed three times weekly. Identification was made macroscopically by color of the colony's top and reverse, surface characters and margin shape and microscopically by adhesive tape preparation[7].

Culture on Dermatophyte Test Medium (BBL): The medium was prepared according to manufacturer's instructions; the inoculated plate was incubated at room temperature aerobically for up to 14 days. The plate was examined daily for development of a red color; most pathogenic dermatophytes produced color change in 7 - 10 days. Appearance of white aerial hyphae and pink to red color around the fungal growth indicated positive dermatophyte result but growth without color conversion to red denotes that the organism is not a dermatophyte [8].

Molecular detection of dermatophytes by pan-dermatophytes nested PCR:

DNA extraction: DNA was extractd automatically on the QIAcube (Qiagen) and the concentration of DNA was detected by Nanodrop (Thermo scientific).

Primers (Invitrogen): using pan-dermatophytes primers targeting the *CHS1* gene shared by the three genera, i.e., *Trichophyton*, *Microsporum*, and *Epidermophyton*[9].

First-round PCR:

CHS1 1S (5'-CATCGA GTA CAT GTG CTC GC-3')

CHS1 1R (5'-CTC GAG GTC AAA AGC ACG CC-3)

Nested-PCR:

JF2 (5'-GCA AAG AAG CCT GGA AGA AG-3')

JR2 (5'-GGA GAC CAT CTG TGA GAG TTG-3')

PCR amplification: using Taq PCR Master Mix (Qiagen) and bands at 288 bp were taken as positive for dermatophytes.

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages. Z test for independent proportions (Z Prop.) was used to analyze categorical variables. Cohen kappa test was used to assess degree of agreement between the studied techniques. The accepted level of significance in this work was stated at 0.05.

RESULTS

High dermatophyte detection rate (82%) was obtained with nested PCR assay, followed by culture on DTM which was positive in 78 % of cases. Hyphae and/ or spore were observed in 68% of samples by direct microscopy and lastly, dermatophytes were isolated from 56% of samples by culture on either SDA or Dermasel agar with chloramphenicol and cyclohexamide. *Microsporum canis* was the most commonly encountered dermatophyte in 24/56 isolates (42.9%), followed by *T. mentagrophytes* in 13/56 (23.2%) as shown in table 1, figures 1 – 5.

Table 1: Number and percentage of evolved strains in the 56 isolates obtained by conventional culture method

Aetiological agent	Dermasel Agar	
	No.	%
T. mentagrophytes	13	23.2%
T. tonsurans	4	7.1%
T. violaceum	1	1.8%
T. rubrum.	6	10.7%
T. schoenleinii	2	3.6%
T. verrucosum	2	3.6%
M.audouinii	4	7.1%
M.canis	24	42.9%
Total	56	100%

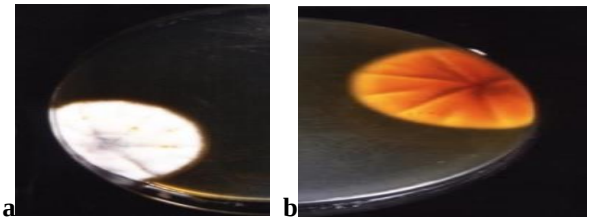

Fig. 1: Top (a) & reverse (b) views of macroscopic colony morphology of *M. canis* on Dermasel agar.

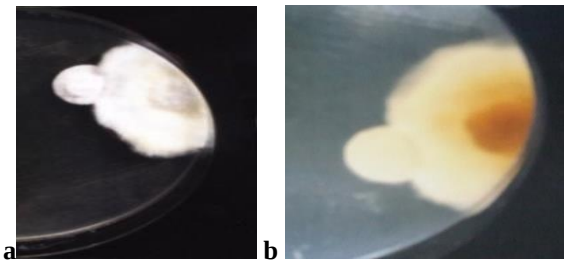

Fig. 2: Top (a) & reverse (b) views of macroscopic colony morphology of *T.mentagrophytes* on Dermasel agar.

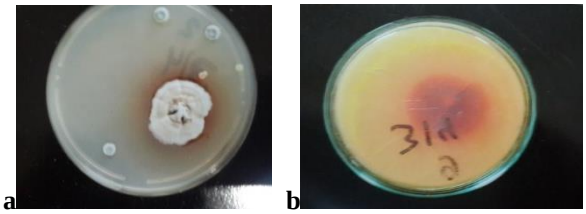

Fig. 3: Top (a) & reverse (b) views of macroscopic colony morphology of *T. rubrum* on Dermasel agar.

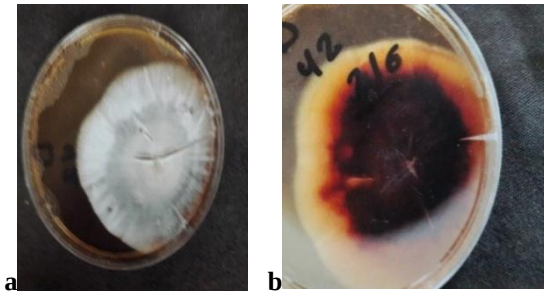

Fig. 4: Top (a) & reverse (b) views of macroscopic colony morphology of *M. audouinii*, on Dermasel agar.

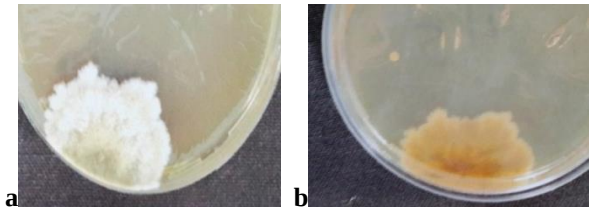

Fig. 5: Top (a) & reverse (b) views of macroscopic colony morphology of *T. verrucosum* on Dermasel agar.

DTM could detect tinea capitis with higher frequency than that of KOH microscopy and conventional cultures (78 versus 68 & 56 respectively) with first appearance of growth for most of the isolates within 10 days of inoculation as shown in figure 6. Dermatophytes metabolize protein in the culture medium first, releasing alkaline metabolites that change the color of the media from yellow to red at the same time the dermatophyte colony appears. None-dermatophyte can turn the color of the medium to red this usually occurs several days after the fungal colony appear.

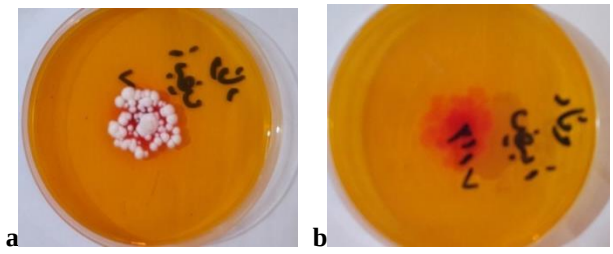

Fig. 6: Top (a) & reverse (b) views of inoculated DTM showing positive result for dermatophyte

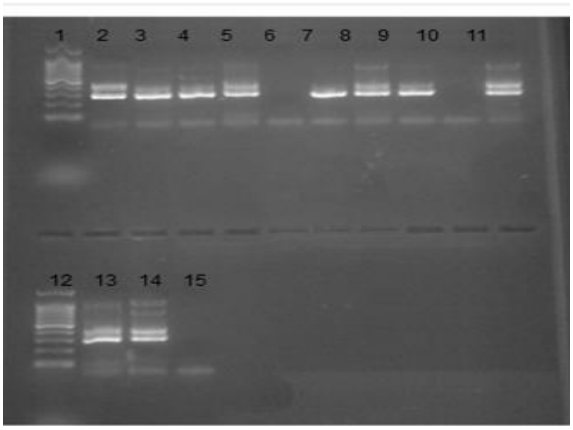

Fig. 7: Band at 288 bp was considered positive for dermatophytes

In evaluation of concordance, there was good agreement between nested PCR and direct microscopy for detection of dermatophytes (Kappa test=0.636, P<0.001) as shown in table 2 plus moderate agreement between PCR and conventional culture (Kappa test=0.437, P<0.001) as shown in table 3. Nearly similar results was obtained with DTM, as there was good agreement between DTM and direct microscopy (Kappa test=0.749 - P<0.001) as shown in table 4 besides moderate agreement between DTM and conventional culture (Kappa test=0.528, P<0.001) as shown in table 5. Nevertheless, there was very good agreement between the two investigated methods (96% - Kappa test = 0.875, P < 0.001) as shown in table 6 and our data indicated that the two assays yielded higher positivity in less time than conventional methods.

Table 2: Concordance between nested PCR & KOH microscopy for diagnosis of tinea capitis

			KOH		Total
			Negative	Positive	
PCR	Negative	Count	18	0	18
		% within KOH	56.2%	.0%	18.0%
	Positive	Count	14	68	82
		% within KOH	43.8%	100.0%	82.0%
Total		Count	32	68	100
		% within KOH	100.0%	100.0%	100.0%

Kappa test=0.636, P<0.001 (HS), degree of agreement=86%

Values of Kappa (0.2=Poor, 0.21-0.4= Fair, 0.41-0.6= Moderate, 0.61-0.8 = Good, 0.81-1.00=Very good).

Table 3: Concordance between nested PCR and conventional culture for diagnosis of tinea capitis.

			SDA		Total
			Negative	Positive	
PCR	Negative	Count	18	0	18
		% within SDA	40.9%	.0%	18.0%
	Positive	Count	26	56	82
		% within SDA	59.1%	100.0%	82.0%
Total		Count	44	56	100
		% within SDA	100.0%	100.0%	100.0%

Kappa test= 0.437, P<0.001 (HS), degree of agreement=74%

Table 4: Concordance between DTM and KOH microscopy for diagnosis of tinea capitis

			DTM		Total
			Negative	Positive	
KOH	Negative	Count	22	10	32
		% within DTM	100.0%	12.8%	32.0%
	Positive	Count	0	68	68
		% within DTM	.0%	87.2%	68.0%
Total		Count	22	78	100
		% within DTM	100.0%	100.0%	100.0%

Kappa test=0.749, P<0.001 (HS), degree of agreement=90%

Table 5: Concordance between DTM and conventional culture for diagnosis of tinea capitis

			SDA		Total
			Negative	Positive	
DTM	Negative	Count	22	0	22
		% within SDA	50.0%	.0%	22.0%
	Positive	Count	22	56	78
		% within SDA	50.0%	100.0%	78.0%
Total		Count	44	56	100
		% within SDA	100.0%	100.0%	100.0%

Kappa test=0.528, P<0.001 (HS), degree of agreement=78%

Table 6: Concordance between nested PCR and DTM methods

			DTM		Total
			Negative	Positive	
PCR	Negative	Count	18	0	18
		% within DTM	81.8%	.0%	18.0%
	Positive	Count	4	78	82
		% within DTM	18.2%	100.0%	82.0%
Total		Count	22	78	100
		% within DTM	100.0%	100.0%	100.0%

Kappa test=0.875, P<0.001 (HS), degree of agreement=96%

The age of the 82 positive cases ranged from 4 -11 years with the mean age of 6.4 ± 2.2 years, 52 were males and 30 females. The most frequent affected age group was from 4 to less than 6 years (42.7%), followed by the age group from 6 to less than 9 (39.0%) and lastly the age group from 9 to 11 years (18.3%). The relation between positive cases in age group (a) to positive cases in age group (b) was statistically insignificant (P>0.05) but the relation between positive cases in age group (a) to positive cases in age group (c) was statistically highly significant (P< 0.01) and the relation between positive cases in age group (b) to positive cases in age group (c) was statistically significant (P < 0.05) as shown in table 7.

Table 7: Number of tinea capitis positive cases in relation to age groups.

Age	Total	%	Z	P
Group (a): 4 to less than 6years	35	42.7	Z1= 0.37	>0.05
Group (b): 6 to less than 9 years	32	39.0	Z2 =2.83	<0.01
Group (c): 9 to 11 years	15	18.3	Z3 =2.48	<0.05

Males were more affected than females in all age groups (63.4% versus 36.6%) with male to female ratio of 1.7 : 1 but this was of no significant statistical value (P > 0.05). 87.8 % (72/82) of positive cases came from rural areas, 45 males (62.5%) and 27 females (37.5%) (P < 0.05), and 12.2% (10/82) lived in urban areas, 7 males (70%) and 3 females (30%) (P > 0.05). These results reveal that peoples in rural areas consequently had higher risk of infection than urban population.

Scaly type tinea capitis was the most frequent clinical type encountered (54.3 %) followed by black dot type (40.2%), then kerion and favus (3.6 & 1.9% respectively) (P > 0.05).

DISCUSSION

Tinea capitis means infection of the scalp by dermatophytes, which cause the disease by their ability to degrade keratin. Overcrowding, low standards of living, poor hygiene along with high humidity are causal factors for the increased prevalence of these infections [1].

Reactions to dermatophyte infection range from mild to severe. The degree of severity depends on a variety of factors such as the virulence of the infecting species, reaction of the host to metabolic products of the fungus, anatomical location of infection and environmental factors [2].

The causative agent cannot be determined in all children depending on clinical features alone and the laboratories may not be equipped for their identification. The prevalence of one agent or other differs depending on the geographic area, migratory movements, hygienic habits, introduction of new treatments and availability of medical resources [10].

In the present study, fungal elements were observed by KOH mount in 68 (68%) sample from patient clinically prediagnosed as tinea capitis. This result was in accordance with that of Garg et al. [9] who reported that (63.8%) of samples were positive for dermatophytes by 20% KOH microscopy. On the other hand, Kansra et al [1] (51%) and Brilhante et al. [11] (48%) reported lower percentage than that detected in our study; this may be explained by the use in our study of samples under more selective conditions than other studies.

Efficacy of SDA with and without chloramphenicol and cyclohexamide for detection of dermatophytes in our study was equal (56% for each) which is near to that detected by Lopes et al [12] (56.6%), but lower percentage was reported by Brilhante et al [11] (36%) and Noronha et al [13] (40%). Conventional culture methods, though specific and sensitive, have two important drawbacks, cultivation needs a long incubation period (up to 4 weeks), and fungal strains may develop abnormal characteristics (atypical colony morphology), in addition, saprophytic fungi can lead to false negative results.

DTM can be used as a simple way for detection and identification of dermatophytes in clinical samples. The medium includes a pH indicator which changes the color of the medium from yellow to red under alkaline condition accompanying growth of dermatophytes and typical colony morphology will be observed [14]. In this study DTM could detect dermatophyte in a higher frequency than that detected by KOH mount and conventional cultures (78 versus 68 & 56 respectively) with first appearance of growth for most of the isolates within 10 days of inoculation. DTM could be used as a screening test for detection of dermatophytes and it is ideal for hospital use but further testing may be required for species identification.

In the previous few years several molecular methods have been developed for the detection and identification of dermatophytes from clinical samples. Major difficulties encountered PCR methods are that it requires sophisticated equipment, personal training and it is also expensive [15]. On the contrary, it is not only sensitive and specific, but also has the likely to decrease the time needed for identification of pathogens that grow slowly or are difficult to culture.

This study investigated the efficacy of nested PCR for detection of dermatophytes in one hundred children pre-diagnosed clinically as tinea capitis. Nested PCR was positive in 82 % of samples (82/100) and this result was in agreement with Garg et al. [9] who reported that dermatophytes were detected in 87 out of 105 patients (82.8%) by nested PCR and also in harmony with the results of Wisselink et al [16] who noticed a significant increase in detection rate for dermatophytes in clinical samples by molecular methods compared to culture.

Nested PCR is a promising tool for detection of dermatophytes in hair samples, providing clinicians with a rapid diagnosis. The use of PCR technology directly on clinical samples will allow early and precise identification of agents of dermatophytosis within 24 hours in comparison to the 4 weeks needed by culture; this would permit prompt and targeted initiation of antifungal therapy [17].

However, limitations of our molecular method must be noted. First, this method is not standardized for routine clinical laboratories. Second, has no discriminative power to differentiate various species. Third, is not practical enough for a large number of laboratories that are either small scale or very tightly budgeted. Fourth, the use of this method in the daily practices of a microbiological laboratory is limited by the number of steps, so complete automation of the method is required to reduce hands on time and time to result and be applicable in a routine setting.

In this study, samples which were negative by direct microscopy (32/100) were also negative by culture however, 12 cases were KOH positive but negative by culture on conventional media this might be owing to non-viability of fungal elements in these cases.

Fourteen of KOH negative samples (43.8%) and twenty six of negative samples by culture (59.1%) were positive by nested PCR, this was in agreement with Garg et al [9] who reported that (55%) of KOH negative samples and (80%) of negative samples by culture were positive by nested PCR which may be considered gold standard for detection of dermatophytes in patients with dermatophytosis (the positivity rates for the evaluated methods are higher than the reference method).

It is necessary that good laboratory method is available for rapid and accurate identification of dermatophytes, in order to apply proper treatment and preventive measures, to ascertain the possible source and prevent spreading [17]. Molecular methods, similar to nested PCR used in this study, is a reliable tool in medical science confirmed by the high success rate at precisely identifying different pathogens. For this reason, their use in mycology has been greatly embraced, as this technique will improve the problem of time consuming and other difficulties, encountered by conventional methods and increase sensitivity and specificity of the diagnosis.

In our study, *M. canis* was the most commonly encountered species (42.9%) followed by, *T. mentagrophytes* (23.2%), In agreement with our results, Bassyouni et al [18] conducted a study on 128 patients in

Fayoum, Egypt and found that *M. canis* was the most prevalent dermatophyte (52%), higher detection rate (64.15%) was observed by Doss et al [19] in their study at Beni Suef University Hospitals on 100 children. Also in Europe. Ginter-Hanselmayer et al [20] found sharp increase of the zoophilic dermatophyte *M. canis* with percentages of 90% in Austria and 54% in Germany.

On the other hand, *T. violeceum* was found to be the most commonly isolated dermatophyte (37.5%) in a study done by Hassan et al [21] in Assuit city and in the multicenter study done by El-Khalawany et al [22] in Cairo, Alexandria, and Tanta and found the prevalence of *T. violeceum* was 56.9%.

These results suggest that the primarily isolated organism differs in different geographic areas and inside a given area during different periods, this may be explained by many factors comprising, weather, socioeconomic conditions, the virulence of the organism, immune status of the hosts and access to treatment.

The age of the positive cases ranged from 4 -11 years with the mean age of 6.4± 2.2 years, they were 52 males and 30 females, 72 lived in rural areas and 10 lived in urban areas.

The most frequent affected age group in our study was from 4 to less than 6 years (42.7%), followed by the age group from 6 to less than 9 years (39.0%) and lastly the age group from 9 to 11 years (18.3). These results go in agreement with the study of Bagyalakshmi et al [23] who reported that, 87.8% occurred in children younger than 10 years of age. Also, Brilhante et al [11] reported that, there was greater number of positive cases of dermatophytes in children under the age of 10 years (n= 309) than in 11–20-year-olds (n= 65) and 21–30-year-olds (n= 19). High occurrence of tinea capitis in children less than 10 years of age may be due to lack of secretion of fungistatic sebum by scalp before puberty.

In this study, males were more affected than females in all age groups (63.4% versus 36.6%) with male to female ratio of 1.7 : 1 but this was of no significant statistical value (P > 0.05). Male dominance may be explained by greater outdoor physical activity and bigger chance for exposure to infection than females. A male child has more contact with animals, and rural boys help out on farms. Also, tinea capitis is more evident in boys with short hair, and in Islamic countries, girls are less infected probably because when they become older they keep their hairs covered, in accordance to Islamic law [24].

In the present study, 87.8 % of positive cases came from rural areas and 12.2% lived in urban areas and these results reveal that peoples in rural areas consequently had greater risk of infection than urban inhabitants. In accordance with our results, Michaels and Del Rosso [25] reported that tinea capitis occurs mainly in rural or suburban areas of Slovenia and the incidence was 77.9%. Several factors associated with this increased frequency in rural areas include poor personal hygiene, overcrowding, illiteracy and low socioeconomic level. In Egypt, these results were also in agreement with Abdel Hafez et al [26] & Moubasher et al [27] they reported that the incidence of tinea capitis in rural areas of Assuit and Sohag Governorates were 86.9% & 85.4% respectively.

In our study, scaly type tinea capitis was the most common clinical type encountered (54.3 %), followed by black dot type (40.2%), then kerion and favus with frequency of 3.6 & 1.9% respectively. Morar et al [28] also reported the scaly type to be the most common clinical presentation seen in 50% of patients. Other investigators stated black dot to be the most frequent type [29]. This variation may be owing to different infecting fungi and different geographic location.

CONCLUSION

By the use of nested PCR, rapid reliable results were achieved within 24 hours in contrast to the 4 weeks of incubation required by conventional culture. This technique is not only rapid but also simple and inexpensive in comparison to other molecular methods and can be considered the gold standard for detection of dermatophytes. DTM could detect tinea capitis with higher frequency than that of KOH microscopy and conventional cultures. It is ideal for hospital practice, can be used as a screening test in locations where specified training and microscopic examination is not accessible but additional testing may be needed for species identification.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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