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Comparative Study between Microscopic Examination and Cultural Media in Diagnosis of Dermatophytosis among School Age Children

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Abstract

Introduction: Dermatophytes are among the common fungal agents implicated in superficial skin infections worldwide. The difficulty in the diagnosis of dermatophytosis relies primarily on the absence of standardization of the collection of clinical specimens and of mycological techniques. Direct examination is essential, as it allows the clinician to start treatment, pending culture. The present study was undertaken with comparative evaluation of microscopic examination and culture media for fungal isolation Methods: Seventy five clinically suspected cases of dermatophytosis were subjected to mycological examination with microscopy and culture using 10% KOH and Sabouraudâ€™s Dextrose Agar (SDA) with cycloheximide. Results: Direct microscopy detected fungal elements in 37 samples from 38 showed positive by culture, the 37 sample which showed negative result by culture method, only 11sample showed negative result by direst microscopy. Conclusion: Difficulty in the diagnosis of dermatophytosis is due to absence of mycological techniques and standardization of collection clinical specimens in addition to lack of commercial availability for most of the reagents needed. Our result and other founding else were created the possibility to clinician to rely on direct microscopical result for treatment of dermatophytosis.

Keywords: Dermatophytosis, Microscopic Examination, Cultural Media.

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Introduction

Dermatophytosis, au superficial cutaneous fungal infection, It is an infection of the skin , hair or nails by any of a group of keratinophilic fungus which are classified into three genera based primarily on differences in microscopic morphology and modes of sporulation as - Epidermophyton, Mirosporum and Trichophyton. (1) The organisms colonize the keratin tissues and inflammation is caused by host response to metabolic by-products.

They are assuming greater significance both in developed and developing countries particularly due to advent of immunosuppressive drugs and disease. (2) Hot and humid climate in the tropical and subtropical countries like Sudan makes dermatophytosis or ringworm a very common superficial fungal skin infection.

Dermatophytes are transmitted by direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in clothing, combs, hair brushes, theatre seats, caps, furniture, bed linens, shoes, socks, towels, hotel rugs, sauna, bathhouse, and locker room floors. (3)

The difficulty in the diagnosis of dermatophytosis relies primarily on the absence of standardization of the collection of clinical specimens and of mycological techniques, but also on the lack of commercial availability for most of the reagents needed. (4)

Direct examination is essential, as it allows the clinician to start treatment, pending culture. Although false-negative results have been reported in 5–15% of cases in routine practice, depending

essentially on the skill of the observer and on the quality of sampling, this is a highly efficient screening technique [5].

Culture is a valuable and often obligate complement to direct examination. Indeed, isolation of the pathogen by culture and its identification at the species level which cannot be reached by direct examination or histopathology, are important since prophylaxis and therapy may vary depending on the species.

Moreover, in case of onychomycosis or tinea capitis, the confirmation of the diagnosis can also be useful in motivating the patient to undertake the often lengthy treatment [6].

The present study was undertaken with comparative evaluation of microscopic examination and culture media for fungal isolation.

Materials and methods

Seventy five samples were collected from clinically suspected cases of dermatophytes infection between July and August 2012, attending the outpatient department of Skin and Venereal disease department of Khartoum Teaching Hospital, Khartoum state, Sudan.
In the presented study male patients were 38 and females patients were 37. The 75 samples consisted of 40 samples hair, 30skin and 5nails samples

Collection and transportation of specimens:
Suspected lesions were cleaned with 70% alcohol to remove the dirt and contaminating bacteria. Samples were collected in sterile paper, folded, labeled and brought to the laboratory for further processing.

Skin scales were collected by scraping the surface of the margin of the lesion using a sterile scalpel blade .Crusts were collect by removing part of the crust nearest to healthy skin using sterile scissor and tweezers. Nail pieces were collect by taking snipping of the infected part of the nil using sterile scissors. Hairs were collect by removing dull broken hairs from the margin of the lesion using sterile tweezers. After collection of the specimens, the paper was folded to form a flat packet. Then it was labeled with the patient's name and number [7].

For direct microscopy samples examination was done using 10%KOH solution to see hyphal segments in skin scales or either ectothrix or endothrix invasion of infected hairs and Nils . A drop of 10% KOH was kept on a clean, grease free glass slide. The sample (hair, skin and nail clipping) was placed in the KOH drop; samples was kept in 10% KOH for a variable duration ranging from 5 minutes to 30 minutes, depending upon the thickness of the scales and examined every 5 minutes.

For primary isolation of dermatophytes Sabouraud Dextrose Agar (SDA) with cycloheximide which inhibit the overgrowth of bacteria and prevent the overgrowth of the rapidly growing saprophytic fungi were used as follow: Skin scales, crusts and pieces of nails were cut into pieces as small as possible using a sterile blade. The small pieces of specimens were inoculated using sterile tweezers on the surface of a plate of malt agar.

Hairs samples were cut using sterile scissors into small pieces (portion nearest to the hair root) about 3-5 mm long. The pieces were inoculated using sterile tweezers on the surface of a plate of malt agar.

The plates were incubated at room temperature (25-30°C) and examined at intervals for evidence of fungal growth. Plates not showing growth for four weeks were discarded. Any visible growth from SDA was examined for colony morphology, texture, surface pigmentation and pigmentation on the reverse.
Microscopic examination of colony was done by doing a lactophenol cotton blue mount to examine the hyphal structure, microconidia and macroconidia.

Result

Seventy five cases of clinically suspected dermatophytosis were subjected to mycological examination. In the presented study male patients were 38 positive fungal elements found in 30 (78.9%) patients and female patients were 37 and positive fungal found in 33(89.2%) (table.1).

Table.1 Results in relation to gender

		Gender				**Total**	
		Male		Female			
		F	%	F	%	F	%
Microscopic examination results	Positive	30	78.9	33	**89.2**	**63**	**84.0**
	Negative	8	21.1	4	**10.8**	**12**	**16.0**
Total		**38**	**100.0**	**37**	**100.0**	**75**	**100.0**

Different specimens were collected including, hair 40(53.3%), skin 30(40%) and nails 5(6.7%) samples. Hair samples showed positive culture result in 18 (24.0%), skin samples showed in 20 (26.7%), while all 5 Nils samples showed negative result (table.2).

Table .2 Types of samples in relation to culture examination results

		Culture result				**Total**	
		Positive		Negative (contaminated)			
		F	%	F	%	F	%
Samples	Hair	18	24.0	22	29.3	**40**	**53.3**
	Skin	20	26.7	10	13.3	**30**	**40.0**
	Nails	0	0.0	5	6.7	**5**	**6.7**
Total		**38**	**50.7**	**37**	**49.3**	**75**	**100.0**

Direct microscopy revealed fungal elements among hair samples in 34 (38.7%), skin samples in 29 (45.3%) while all skin sample showed negative result (table.3).

Table.3 Types of samples in relation to microscopic examination results

		Microscopic examination results				Total	
		Positive		Negative			
		F	%	F	%	F	%
Samples	Hair	34	45.3	6	8.0	**40**	**53.3**
	Skin	29	38.7	1	1.3	**30**	**40.0**
	Nails	0	.0	5	6.7	**5**	**6.7**
Total		**63**	**84.0**	**12**	**16.0**	**75**	**100.0**

Culture technique isolated 38 (50.7 %) fungi, while direct microscopy revealed fungi in 63 (84.0%) samples (table.4).

Table.4 Results of culture and direct microscope examination

	Positive		Negative		**Total**	
	F	%	F	%	F	%
Microscopic direct examination	63	84.0	12	16.0	**75**	**100.0**
Culture	38	50.7	37	49.3	**75**	**100.0**

Direct microscopy detected fungal elements in 37 samples from 38 isolated by culture, on the other hand from 37 sample showed negative result by culture method, only 11sample showed negative result by direst microscopy (table.5).

Table .5 Microscopic results in relation to culture results

		Culture result				**Total**	
		Positive		Negative (contaminated)			
		F	%	F	%	F	%
Microscopic examination results	Positive	37	49.3	26	34.7	**63**	**84.0**
	Negative	1	1.3	11	14.7	**12**	**16.0**
Total		**38**	**50.7**	**37**	**49.3**	75	**0**

Isolated fungi were identify as, *Trychopytone rabrum* 45(65.2%), *Trychophytone sp (Sudanese)* 15(21.7%), *Trychophytone violacum* 5(7.2%), and *Micropsporeum canis* 4(5.8%) (table.6).

Table .6 isolated fungi

		Frequency	%
Identified species by culture	*Trychophytone rubrea*	45	65.2
	Trychophytone sp (Sudanese)	15	21.7
	Trychophytone violacum	5	7.2
	Micropsporeum canis	4	5.8
Total		**69**	**100.0**

Discussion

Among the various fungal infections of human beings dematophytes is a most common infection of the world.[8]. They are assuming greater significance both in developed and developing countries particularly due to advent of immunosuppressive drugs and disease. Hot and humid climate in the tropical and subtropical countries makes dermatophytosis or ringworm a very common[9].

Laboratory diagnosis of dermatophytes always was the problem in developing country like Sudan, lack of facility and training staff of diagnostic fungal specialist with time cost of the routine culture technique lead worker to looking for microscopic examination of dermatophytes as alternative technique. This study designed to assess the efficient of microscopic examination of dermatophytes by comparing it with routine culture on Sabouraud dextrose agar (SDA).

In the presented study isolated fungi among male patients were 30 (78.9%) and female patients were 33(89.2%).Our result almost agree with study done with many studies that showed no significant difference of dermatophytosis among males and females as in Kuwait [10] and Mexico [11], however, there are many studies showed higher infection among males [12, 13, 14] and others showed more infection among females [13, 14]. The reason for these variations is not fully understood, but it indicates that gender may influence susceptibility to a particular form of tineas [15].

Direct microscopy revealed fungal elements in (84.0%), of these 37(49.3%) were culture positive. culture showed fungal growth in 38 samples, only one case was negative on direct microscopy. out of 75 samples studied 11 (14.7%) did not show evidence of the fungi either on direct microscopy or culture. Our result in agree with Bindu and his colleague which they observed in their study that in direct microscopy positivity was 64% cases and culture positivity was 45.3% cases [16].

S, Singh, P.M. Beena in 2003 also agree they reported 60.38% cases positive by microscopy and 44.6% cases were culture positive. 53.38% cases did not showed evidence of fungus either on direct microscopy or on culture [17].

Other study done in india by SS Sen, ES Rasul in 2006 disagree with our result, they reported that 4.9% cases were positive for

fungal elements by direct microscopical examination, culture was positive in 51% cases[18].

Trychopytone rubrum was the predominant isolate accounting for (50%), *Sudanese Trychophytone* (28.9%), *Violacum sp* (10.5%), and *Micrsporium sp.* (10.5%), this result in agree with two Indian study done by Sumana and his colleagues in 2004 and Sen and his colleague in 2006[18, 19].

Another study by different workers also in agree with our result, they founded that, *T.rubrum* as predominant isolate in their studies, were Sumit Kumar et al in 2014 – 65.09%. [20] Mohanthy JC et al in 1998 – 68.34%, [21] Bindu V et al in 2002 [22], Sumana V et al in 2004 – 60%[19], Peerapur B V et al in 2004 – 43.7%[23], Gupta BK et al in 1993 -42.42%. [24]

Conclusion: Difficulty in the diagnosis of dermatophytosis is due to absence of mycological techniques and standardization of collection clinical specimens in addition to lack of commercial availability for most of the reagents needed. Our result and other founding else were created the possibility to clinician to rely on direct microscopical result for treatment of dermatophytosis.

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