Original Article

Epidemological and Molecular Study of patients infected with scabies (Sarcoptes scabiei) in Thiqar Province, Iraq

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Abstract

The aim of the present study was to epidemiological and molecular detection of patients infected with scabies caused by Sarcoptes scabiei during the period from January2018 to December in the same year in Iraq, Thiqar Province. A total of 721 patients were infected with scabies, 386 (53.5%) were male and 335 (46.5%) were female .Age group 15-45 y old was the most prevalent group (220 patients, 30.5%) infected with scabies. August t 2018 was the highest month in infection (107 patients, 14.8%) and December was the lowest month in infection (20patients, 2.8 %).Students196(27.20%) were the highest percent while the lowest percent of patients were in Crippling 4 (0.6%). However, most such studies have involved epidemological, and limited molecular diagnostic studies. The aim of this study was the genetic characterization of S. scabiei 20 samples from patients ,this study has been the first time in Thi-Qar province . Molecular identification was performed this study in the Laboratory of College of Science, University of Thiqar, using two primers COX and scabie primer . The results showed with agarose gel electrophoresis revealed that 250 bp and 289bp respectively . Visualized under U.V light after staining with Ethidium bromide

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Keywords: Scabies, Epidemiology, Molecular identification, Thiqar , Iraq.

INTRODUCTION

Scabies is one among the foremost important skin diseases worldwide and a common between human and animal because it is characterized by crusted lesions and causes severe itching and dermatitis also as loss of hair and feathers additionally to loss of appetite (Elgart, 1990).

The main explanation for this disease may be a very small mite Sarcoptes scabiei and is one among the ectoparasites infected ,each year around 300 million people worldwide are suffering from scabies (Hengge et.al.,2006).

Cases of scabies still spread sporadically in hospitals, elderly homes, prisons and schools where close contact and exchange of furniture and clothing also due to the incidence of infection in Slums and military barracks also are scattered in crowded places that lack good hygiene (Bouvresse and Chosidow, 2010; Stoevesandt et al., 2012)

In Iraq, many studies about the scabies by Al-Samarai, (2009)who study prevalence of scabies was high during a dermatology clinic, in Tikrit province, he was found that the infection were more common in children.

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Other study in Tikrit Teaching Hospital, that study showed this disease affects males (53%), females (47%), were more common in children's and adults than elderly (Najem et al., 2009). in Karbala city , study at Al-Hussein Teaching Hospital, scabies account for 6.86 percent of all skin diseases in patients who have visited dermatology clinics and outpatient clinics. (Al-Mendalawi and Ibrahim, 2012). In Baquba city, Alzobydy (2018) study the prevalence of scabies infection was (2.16%) among patients with different disease of the skin (1,5891) and fewer prevalent in females (37.8%) than male (62.2%) and highest prevalence was observed in age bracket quite (13) years(77.3%) . Other study in Al-Najaf province by Mohy et al., (2018) obtained high numbers of people infected with scabies especially in rural areas. In Thi Qar province , there's limited previous studies about scabies in Thi-Qar province .Therefore, this research was designed to study the epidemological and molecular identification of scabies with patients during 2018 year.

Materials and Methods

1- Patients :

This study was conducted in Thi Qar province, Iraq. A total of (721) patients (male and female) infected with scabies were included in this study during the period from the beginning of January to December 2018.

Some information were taken from patients (age, gender , presence of animals) age ranges between less than 1 year to more than 45 years.

All patients infected with scabies caused by Sarcoptes scabiei were diagnosed consistent with physical exam examination and checked the affected region of skin or removing a S. scabiei with a needle from the skin and scraped off a small section of skin to get a tissue sample and examined this sample under a microscope to check the presence of scabies mites or their eggs (Chosidow,2006) .

2-Collection and storage of Sarcoptes scabiei

1-Fixation with ethanol:

The Sarcoptes mites have been isolated from scabies patients. Scarpings were taken from clinically leishions that were clinically suspicious .Each specimen was placed in a sterile plastic bottle and collected samples were fixed in 100 % ethanol and stored separately at 4C^{o} before DNA extraction as described by Berrilli et al.,(2002).

2- Viral transport media:

For patients with crusted scabies, skins swabs were additionally taken at the same time as skin scrapings for PCR. The six body sites which are most often affected by scabies, viz. scalp, finger web, wrist, elbow, popliteal fossa and ankle, were taken from swabs. The tip of the swabs was placed in the viral transport medium which were then stored at 4°C for a maximum of three days before nucleic acid extraction. For the ease of preserving skin scrapings in solution form, viral transfer medium was used and it avoids sample degradation due to overgrowth of bacteria or fungi.

3-DNA Extraction from skin's tissues samples

DNA extraction was performed using kit (geneaid, Taiwan) according to manufacturer's instructions.

https://www.geneaid.com/-Genomic-DNA-Purification/GS.

4-Preparation of PCR primers

The primers are prepared depending on the manufacturing instruction by dissolving the lyophilized primers with TE (Tris-EDTA) buffer to make stock solution of concentration of 100 pmole/Ml, primers working solution were prepared by diluting the stock solution with TE buffer to get final working solution (10 pmole/Ml) for each primer.To detect scabies in table 1.

Table 1: Oligonucleotide primers used in this study

Species	target gene	Primer	Primer Sequence (5'-3')	Amplicon size (bp)	References
scabies	COX	COX F COX R	5'- CTTATTATTCCTGGATTTGGRTA -3' 5'- CTAATTTTCCTCCTAATATTGTWGA -3'	250 bp	doi:10.1128/JCM.00073-15
	scabies	ScaBI F ScabI R	5′- TCTTAGGGGCTGGATTTAGTATG -3′ 5′-GAAGCTTTTCACCATTAGAAGCTG	289 bp	DOI: 10.20473/ijtid.v6i6.5436

5-PCR master mix reaction preparation

PCR master mix reaction was prepared by using (one taq quick-load)PCR Kit and this master mix done according to company instructions https://www.neb.com/products/m0486-onetaq-quick-load-2x-master-mix-with-standard-

buffer#Product%20Information

6-Gel electrophoresis

PCR products of multi gene were analyzed by using agrose gel electrophoresis method as following steps:

1- 1% Agarose gel was prepared in using 1X TBE and dissolving in microwave for 2min, after that, left to cool 50°C.

2- Then 2µl of ethidium bromide stain were added into agarose gel solution.

3- Agarose gel solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 6µl of PCR product were added in to each comb well and 2 µl of (1500 bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then electric current was performed at 100 volt for 30 min. then 50 volt for 45 min.

5- PCR products were visualized by using ultraviolet trans illuminator.

4-Statistical analysis: The SPSS statistical program was used V.22 to do statistical analysis using Chi-square (X2).

Results

- Epidemological Study

-Distribution of patients infected with Scabies according to gender and age groups

Out of 721 patients(386 male and 335 female)the results of the statistical analysis showed that there are significant differences between the age groups of males and females, the results show in the table (2) the difference in percent of infection according to age stages, age group15-45y old was the most prevalent group 377(52.3%) infected with scabies ,while the lowest prevalence was seen in the age group less one year 22(3.1%),and the other age groups 1-4, 5-14 and ≤ 45 y old were 57(7.9%) 156(21.6%), and 109(15.1%) respectively.

Table (2): Distribution of patients according to age groups and gender

Age groups	Patients				Total	Percent
	Male	%	Female	%		
≥ year	13	1.8	9	1.2	22	3.1
1-4	28	3.9	29	4	57	7.9
5-14	85	11.8	71	9.8	156	21.6
15-45	220	30.5	157	21.5	377	52.3
≤ 45	40	5.5	69	9.6	109	15.1
Total	386	53.5	335	46.5	721	100
Statistical analysis		**X^2=16.721**		**DF=4**	**Sig.0.002**	

-Distribution of patients infected with Scabies according to months and gender

The highest month infection was August recorded 107(14.8%) ,were males 59(8.2%)and 48(6.7%) were females, while the lowest month in infection was December recorded 20 (2.8%) distributed between males and females11(1.5%) and 9 (1.2%) respectively. The results showed in table (3).

Table (3): Distribution of patients infected with Scabies according to months and gender

Months	Patients				Total	Percent%
	Male	%	Female	%		
Jan.	26	3.6	21	2.9	47	6.5
Feb.	16	2.2	24	3.3	40	5.5
Mar.	23	3.2	25	3.5	48	6.7
Apr.	43	6	34	4.7	77	10.7
May.	55	7.6	39	5.4	94	13
Jun.	46	6.5	31	4.3	77	10.7

Jul.	37	5.1	22	3.1	59	8.2
Aug.	59	8.2	48	6.7	107	14.8
Sept.	23	3.2	30	4.2	53	7.4
Oct.	33	4.6	27	3.7	60	8.3
Nov.	15	2.1	24	3.3	39	5.4
Dec.	11	1.5	9	1.2	20	2.8
Total	387	53.7	334	46.3	721	100
Statistical analysis		**X^2=13.837**	**Df=11**		**Sig.=0.242**	

-Distribution of patients infected with Scabies according to occupation and gender

The statistical analysis showed that there are significant differences in patients infected with scabies at P≤ 0.05 according to the occupation we found the highest percent of patients were in students196(27.20%) followed by not employed 156(21.6%) ,while the lowest percent of patients were in crippling 4 (0.6%) the percent of infection reached 5 (0.7%) each one in babies , prisoners and retired .Mentioned in table (4).

Table (4): Distribution of patients infected with Scabies according to the occupation and gender

The occupation	**Patients**				**Total**	**Percent%**
	Male	**%**	**Female**	%		
Older	7	1.0	21	2.9	28	3.9
babies	4	0.6	1	0.1	5	0.7
Children	60	8.3	52	7.2	112	15.5
Crippling	2	0.3	2	0.3	4	0.6
Employed	38	5.3	12	1.7	50	6.9
Gainer	146	20.2	0	0	146	20.2
Not Employed	0	0	156	21.6	156	21.6
Prisoners	5	0.7	0	0	5	0.7
Retired	5	0.7	0	0	5	0.7
Soldiers	14	1.9	0	0	14	1.9
Students	106	14.7	90	12.5	196	27.2
Total	387	53.7	334	46.3	721	100
Statistical analysis		X^2= 348.183	DF=10	SIG=0.00001		

- Molecular Study

This study used the polymerase chain reaction PCR method for the molecular identification of Sarcoptic mites for first time in Thi-Qar province,.Analysis of PCR product showed one specific band of 250 bp with primer COX and 289 bp with scabies primer on 1% agarose gel in lane L:DNA lader (1500-100bp) (Fig. 1), (Fig. 2) and (Fig. 3)20 samples of Scabies were extracted DNA from patients .This microsatellite marker is considered to be a standard for the identification of presence of Sarcoptic mites within the skins human samples.

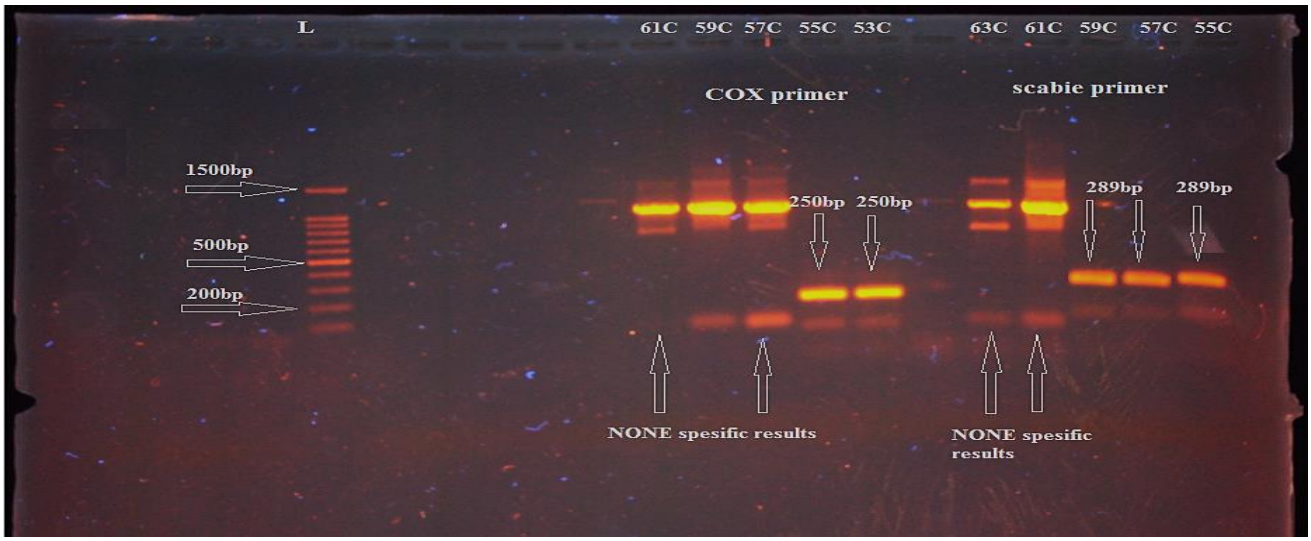

Figure (1):- Gel electrophoresis for optimization process with different temperatures for PCR product of 4 primers (COX and scabie primers) which shows 250bp and 289bp respectively . (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized under U.V light after staining with Ethidium bromide. Lane L : DNA ladder (1500-100)bp .

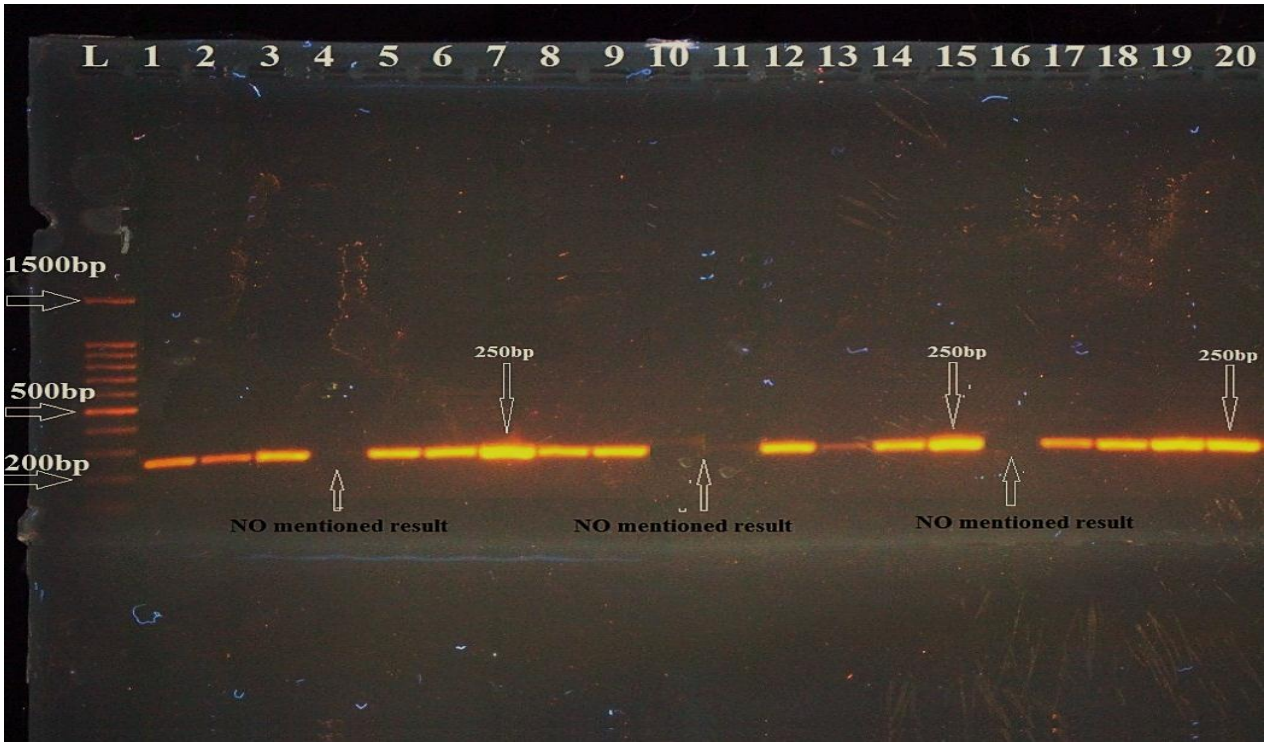

Figure (2): Gel electrophoresis for PCR product of (COX primer) show 250bp Primer TM at (55C). (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized under U.V light after staining with ethidium bromide. Lane L : DNA ladder (100-1500bp), Lanes (1-3),(5-9),(12-15)and (17-23) represented positive results of , lanes(4,10 ,11 and 16) represent negative results .lane N represent negative control

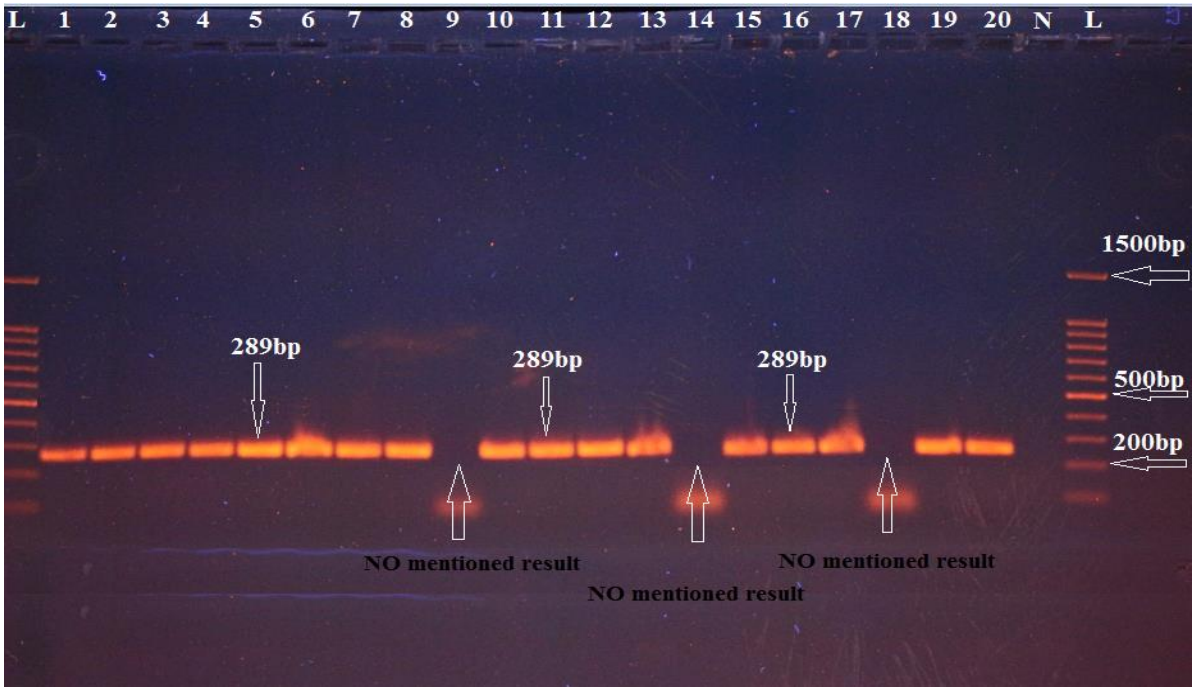

Figure (3): Gel electrophoresis for PCR product of (scabie primer) show 289bp Primer TM at (59C). (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts,60min.).Visualized under U.V light after staining with ethidium bromide. Lane L : DNAladder (100-1500bp), Lanes (1-8),(10-13),(15-17)and (19-20) represented positive results, lanes(9,14 and 18) represent negative results .lane N represent negative control

Disscusion.

The study showed the prevalence and molecular identification of scabies isolated from patients in Thi-Qar province for 2018 year, out of 721patients,(53.5%) were male and (46.5%) were female infected with scabies .Almost ,males were more prevalent than females.

These results are in agreement with some previous studies in Iraq ,by Al-Samarai (2009) in Baghdad province , Iraq ,proved that out of total 11194 patients infected with scabies there were male 13.5% and female were 8.6%. Another study in Baghdad, by Sharquie (2012) who found in his study out of total 97 patients infected with scabies there have been 58 (59.8%) males and 39 (40.2%) females. In Al-Najaf province Al-Mohy et al.,(2018) found 1112 patients, 602 (54.1%) were males and 501 (45.9%) were female. In Baquba City, Alzobydy (2018)found in his study (344) patients diagnosed as scabies (2.16%), they were 130(37.8%) females and 214 (62.2%)males. In Thiqar province by Mousa and Hassan(2020) founded the high percent of scabies patients were in males55.9% and 44.1% in females.

The current result the most common age group infected with scabies was 15-45year old. This result was in agreement with Al-Samarai (2009) in Iraq when he showed that there were 42 patients (31.8%) infected with scabies were among age group 30-44 y .In Thiqar province, previously recorded in paper by Mousa and Hassan(2020) the highest number of scabies patients in the 11-20 age group (22.9%), and the least number for one patient (0.16%) was in the 81-90 age group.

In other studies proved Al-Musawi,et al., (2013) which found age group (10-19) years is the most affected reached 41.7% . age group (6-20) years . This agrees with the work of Al-Dulaimi and Al-Shaha(2015) in Al-Anbar province , whom reported that there was high infection in the age group (pre-school year) , (18-24) and (30-36) year which was (18.40 , 15.50 , 13.80)% respectively.

August and May months were the highest infection (107,94 patients, 14.8 % ,13%) respectively and December was the lowest infection (20 patients, 2.8%).

The current study disagreement with Mohy et al.,(2018) found his study February 2018 was the highest month in infection (187 patients, 16.8%) and May 2017 was the lowest month in infection (25 patients, 2.2%) in Najaf province. It is known that the prevalence of scabies is more common in cold seasons than hot seasons . (Downs, et al.1999; AL-Hadithi and Habash ,2000; Mousa and Hassan,2020)) through the means of transport known within the folds of clothing and covers that are used more intensively on cold days as they were considered a safe hiding place for them, while the results of the current study showed the prevalence of infection in cold and hot seasons, possibly due to the deterioration of the infrastructure and conditions experienced by the Iraqi citizen, as well as the lack of typical sanitary conditions in this part of the country, on the one hand, the presence of animals and insects in crowded and infected populations are factors that

perpetuate the injury and its prevalence on the other hand(AL-Hadithi and Habash ,2000) .

The highest percent of patients were in students196(27.20%) the lowest percent(0.7%) each one in babies, prisoners and retired.

We can explain the result of this study, reported on the rapid means of infection and clearly indicates the transmission of male infection ,This finding supports the rapid means of infection documented in previous studies and clearly indicates the transmission of infection from male to female, due to the movement of males and their constant departure from the home more than females exposed to the sources of infection, attended the dermatological consultation outpatient clinic or sellers hikers, barber shops, laundry shops, clothing stores, etc., all have a role in the events of infection and transmission to males, and epidemiological studies show that the rate of prevalence varies from country to country, as the disease is transmitted between segments of society and citizens as a whole. Their age differences and unspecified times come with opportunities for infection (Downs et.al.,1999 ;Galadari and Sheriff ,2006).

Previous studies about molecular identification of scabies in Iraq are very rare, except previous studies in the world in in Pakistan by Naz et al., (2013) Analysis of PCR of Sarcoptes scabiei mites were isolated from scabies patients and used two primers Sarms 15 F/R on 2% agarose gel in lane 1showed band of 178 bp While, the same analysis with primer 16S D1/D2 on agarose gel showed bands of 460 bp and 600 bp in lane 2 .Other studies in Hong Kong by Wong et al.,(2015) who used method of polymerase chain reaction (PCR) for molecular identification of scabies patients ,for the 29skin scraping samples that were positive for cox1-PCR, sequencing of PCR product showed 98% nucleotide identity with S. scabiei type hominis cox1 gene .

Polymerase chain reaction (PCR) from skin scraping in patients with suspected scabies. PCR was performed on the skin scrapings to target the cytochrome c oxidase subunit 1 (cox1) gene of Sarcoptes scabiei in South Korea by Bae et al.,(2020)

These methods, based on skin scrapings, were successfully used to etiologically confirm the diagnosis of different clinical degrees of sarcoptic. These universal PCR-based diagnosis methods are highly specific, technically sensitive and more accurate.of detection of the scabies even in smallest amount.

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